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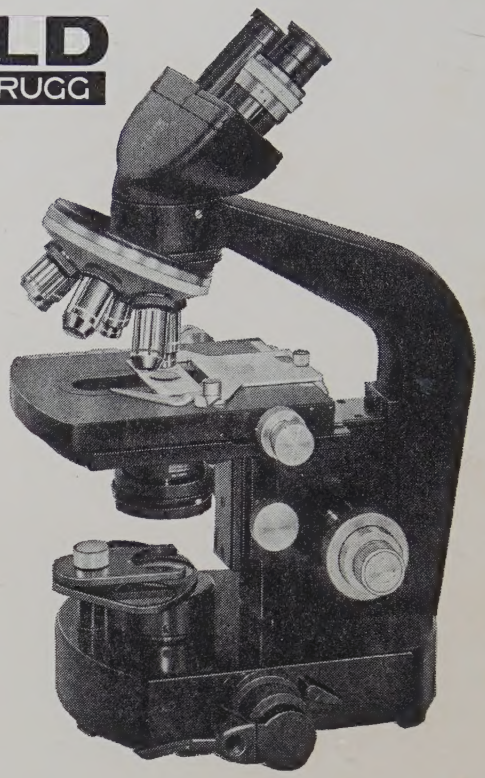

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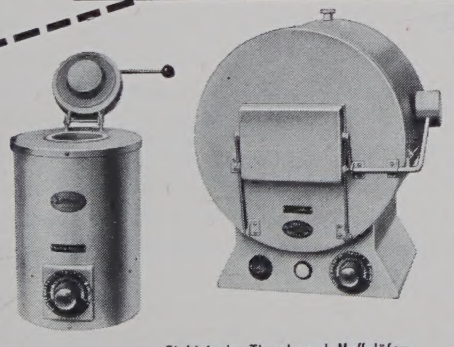

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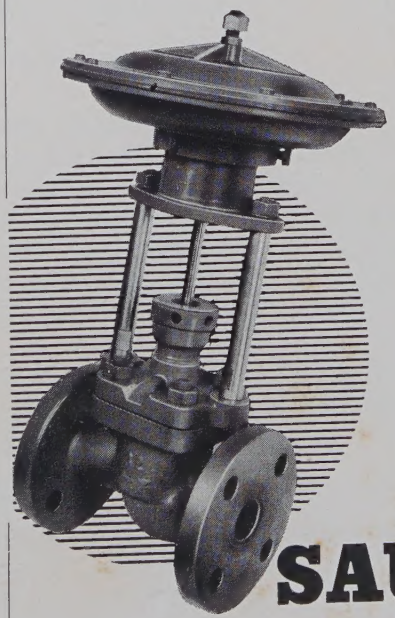

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Sulla struttura microscopica dei cromosomi

Da G. Colombo[1], Padova

Dal complesso delle numerose osservazioni sulla struttura dei cromosomi, che da parecchie decine d'anni si vanno accumulando, alcuni dati si possono ritenere accertati.

I cromosomi sono costituiti da una sostanza esterna, la *matrice*, che avvolge ed include dei filamenti interni, i *cromatidi* o *cromonemi*.

La matrice è principalmente formata da acidi nucleici, proteine basiche e lipoproteine. Nelle varie fasi dei cicli cromosomici la matrice sarebbe la sede di evidenti variazioni metaboliche (Serra[2]). I cromonemi o cromatidi, costituiti di nucleoproteine (Schultz[3]), sono dei filamenti, che in numero vario (1, 2, 4 e forse in taluni casi 8) (Nebel[4]) formano l'asse dei cromosomi e rappresentano l'individualità genetica dei cromosomi stessi.

Le caratteristiche morfologiche, che subito hanno colpito i citologi, sono la torsione del corpo cromosomico, che si vede sopratutto alla profase (Flemming[5]), e la struttura elicoidale dei cromosomi e più particolarmente dei cromatidi, che è visibile anche nella metafase e in alcuni stadi meiotici (Baranetzky[6]).

L'importanza di tale struttura elicoidale è stata messa in evidenza da Fuji[7] e successivamente dai suoi allievi e riconosciuta praticamente per tutti i cromosomi (cfr. Kaufmann, Manton[8]).

L'analisi della struttura microscopica dei cromosomi ha fatto un progresso notevolissimo dopo la diffusione della tecnica dello schiacciamento dopo fissazione e colorazione col carminio acetico (Belling[9]) e successivamente è stata inoltre molto facilitata con l'introduzione della tecnica del pretrattamento delle cellule (con acidi, alcali o semplicemente acqua) prima della fissazione, ideata da Kuwada e Nakamura[1] e modificata successivamente da vari citologi (v. Matthey[2]).

La struttura cromosomica è stata abbastanza ben chiarita con l'esame accurato di materiali favorevoli (specialmente di piante), ma sopratutto con lo studio delle variazioni dell'aspetto dei cromosomi nei cicli mitotici e meiotici.

Le strutture elicoidali secondo Darlington

Dobbiamo a Darlington[3] la più organica descrizione delle modificazioni cromosomiche nel corso dei cicli mitotico e meiotico, interpretate come variazioni di strutture elicoidali dei cromatidi.

Alla profase mitotica, secondo Darlington, si forma un avvolgimento elicoidale («maior coil») dei cromatidi, che man mano si contrae e determina l'accorciamento massimo dei cromosomi alla metafase. All'anafase e alla telofase questa elicoide si svolge.

Lo svolgimento alla telofase e nell'interfase può essere incompleto e nei nuclei interfasici si vedono perciò degli avvolgimenti relitti («relic coils»), che si possono scorgere anche nella successiva profase. Ad ogni profase si instaura un nuovo avvolgimento elicoidale, che non ha alcun rapporto con questi avvolgimenti relitti.

Nella meiosi, secondo Darlington, al leptotene si ha lo svolgimento di elicoidi, che di solito permangono nell'interfase da una mitosi all'altra, la «relic coil» già citata e la «minor coil», di cui avremo occasione di parlare più avanti. Con l'appaiamento cromosomico allo zigotene si avrebbe l'avvolgimento dei cromosomi omologhi tra loro, il quale viene indicato come «relational coil» ed i cui residui sarebbero visibili all'inizio del diplotene.

Dopo il diplotene, alla diacinesi si forma la «maior coil», che porta alla contrazione massima dei cromosomi alla metafase.

Le strutture elicoidali secondo Cleveland

Una recente accurata descrizione ed interpretazione della struttura elicoidale dei cromatidi è quella di

[1] Istituto di Zoologia ed Anatomia comparata dell'Università di Padova.

[2] J. A. Serra, Cold Spring Harbor Symp. quant. Biol. *12*, 192 (1947).

[3] J. Schultz, Cold Spring Harbor Symp. quant. Biol. *9*, 55 (1941).

[4] B. R. Nebel, Cold Spring Harbor Symp. quant. Biol. *9*, 7 (1941).

[5] W. Flemming, *Zellsubstanz, Kern und Zellteilung* (Vogel, Lipsia 1882).

[6] J. Baranetzky, Bot. Z. *38*, 283 (1880).

[7] K. Fuji, 1926, citato da Kuwada e Nakamura.

[8] B. P. Kaufmann, Bot. Rev. *14*, 57 (1948). – I. Manton, Biol. Rev. *25*, 486 (1950).

[9] J. Belling, Biol. Bull. *50*, 160 (1926).

[1] Y. Kuwada e T. Nakamura, Cytologia, Tokio *5*, 244 (1934).

[2] R. Matthey, Rev. suisse Zool. *60*, 225 (1953).

[3] C. D. Darlington, Proc. roy. Soc. London [B] *118*, 33 (1935); *Recent advances in cytology* (Churchill, London 1937).

Cleveland[1], che ha studiato il ciclo cromosomoic mitotico e meiotico in diverse specie di Flagellati. Secondo Cleveland nella mitosi e nella meiosi è possibile riconoscere in seno ai cromosomi un avvolgimento elicoidale costante dei cromatidi, doppi o singoli, che si può osservare in tutte le fasi dei cicli di divisione: la «minor coil».

Questa elicoide è incorporata in un avvolgimento di ordine superiore, la «maior coil», che si forma alla profase e persiste fino alla telofase, rinnovandosi ad ogni ciclo di divisione.

Accanto alla «minor» ed alla «major coil», le quali sarebbero degli avvolgimenti individuali, che interessano cioè i singoli cromosomi ed i cromatidi, vi è, secondo Cleveland, una «relational coil», cioè un avvolgimento dei cromatidi figli tra loro, che si forma in seguito all'autoriproduzione cromatidica. La «relational coil» è presente alla profase e scompare con il contrarsi della «maior coil».

La «relic coil» di Darlington non avrebbe nessun significato strutturale, consistendo semplicemente in un andamento ritorto del corpo cromosomico, residuo della «maior coil» della mitosi precedente.

In complesso le osservazioni e le conclusioni di Cleveland coincidono con quelle di Darlington. Il citologo americano insiste sopratutto sulla costanza della «minor coil», che si osserva bene nei grossi cromosomi dei Polimastigini da lui studiati, e sul fatto che la «relational coil» non rappresenta un vero e proprio stato strutturale dei cromosomi, ma la conseguenza dell'autoriproduzione.

Le descrizioni di Darlington e Cleveland sono probabilmente le più complete ed organiche, ma le ricerche sugli avvolgimenti elicoidali sono numerose (cfr. Kaufmann e Manton[2]) e non tutte concordanti.

«Maior» e «minor coil»

Sulla «maior coil» vi è accordo pressochè generale. È un avvolgimento elicoidale, per lo più a raggio ampio e passo stretto, che si vede bene nei cromosomi profasici e metafasici; ad esso indubbiamente sono dovute le più vistose modificazioni cromosomiche nei cicli di divisione, caratterizzate sopratutto da un forte accorciamento dei cromosomi.

Tale accorciamento, dalla profase alla metafase, è stato accuratamente indagato, per quanto è possibile, anche da un punto di vista quantitativo.

Nella mitosi, dalla profase alla metafase, i cromosomi si accorciano fino a circa $1/3$; nella meiosi, dal pachitene alla metafase della prima divisione, a circa $^1/_{10}$ della lunghezza iniziale (cfr. Darlington[3], Huskins[4]).

Le relazioni quantitative tra accorciamento e grado di avvolgimento in base ad eventuali modelli geometrici non sono facilmente applicabili; le formule elaborate o sono troppo semplificate o richiedono misure, che non si possono prendere con un'approssimazione sufficiente sui preparati microscopici (Manton[1]).

La presenza di un avvolgimento interno dei cromatidi, la «minor coil», è in generale ammessa, benchè, trovandosi questa struttura in molti casi ai limiti della risoluzione microscopica, sia difficile decidere se le immagini che si vedono siano dovute ad un avvolgimento elicoidale oppure ad ingrossamenti disposti lungo l'asse dei cromatidi stessi.

Si considera che l'elicoide «minor» sia quella che si vede chiaramente nella profase e metafase della seconda divisione meiotica e che deriva direttamente dall'avvolgimento interno della prima divisione (Manton[2]). La presenza o meno di una «minor coil» è di fondamentale importanza per la struttura dei cromatidi ed il loro comportamento nelle divisioni dei cromosomi.

Ruch[3] esaminando i cromosomi profasici e metafasici delle cellule madri del polline di *Tradescantia virginica*, dimostrebbe che non vi è doppia elicoide, ma l'aspetto irregolare dei cromatidi, ravvolti secondo la «maior coil», sarebbe dovuto alla presenza di ingrossamenti cromomerici. La presenza di un doppio avvolgimento cromatidico nella mitosi e nella prima divisione meiotica non è completamente dimostrata dalle osservazioni a luce polarizzata di Kuwada e Nakamura[4], che non sono state confermate dalle ricerche di Becker[5].

Cromomeri ed elicoide «minor»

La comparazione delle trasformazioni cromosomiche nella spermatogenesi e nell'oogenesi è molto utile per l'analisi di questo strutture.

Lo studio che ho fatto dell'oogenesi di una cavalletta (*Anacridium aegyptium* L; Catantopinae, Acridoidea, Orthoptera) (Colombo[6]) ed osservazioni precedenti sulla spermatogenesi (Colombo[7]) mi hanno fornito dati interessanti sopratutto sulla questione della presenza o meno dei cromomeri e di avvolgimenti elicoidali.

Per avere un quadro delle trasformazioni nucleari e cromosomiche della meiosi bisogna iniziare l'esame dall'ultima divisione goniale, che in generale è facilmente riconoscibile per le caratteristiche strutturali dei nuclei stessi.

Sopratutto nella spermatogenesi delle cavallette i nuclei dell'ultima telofase goniale hanno una tipica

[1] L. R. Cleveland, Trans. Amer. Phil. Soc. *30*, 1 (1949).
[2] B. P. Kaufmann, Bot. Rev. *14*, 57 (1948). – I. Manton, Biol. Rev. *25*, 486 (1950).
[3] C. D. Darlington, *Recent advances in cytology* (Churchill, London 1937).
[4] C. L. Huskins, Cold Spring Harbor Symp. quant. Biol. *9*, 31 (1941).

[1] I. Manton, Biol. Rev. *25*, 486 (1950).
[2] I. Manton, Bioch. Bioph. Acta *3*, 570 (1949).
[3] F. Ruch, Chromosoma *3*, 357 (1949).
[4] Y. Kuwada e T. Nakamura, Cytologia, Tokio *6*, 78 (1934).
[5] W. A. Becker, Arch. exper. Zellforsch. *22*, 196 (1938).
[6] G. Colombo, Acta Zool. *34*, 191 (1953).
[7] G. Colombo, Exper. *8*, 15 (1952).

struttura a vesicole cromosomiche, descritta già nel 1908 da DAVIS[1].

Questa struttura è dovuta al fatto che permane a lungo la «maior coil» e che i singoli cromosomi mantengono la loro posizione reciproca dell'ultima anafase goniale; ciò probabilmente per il comportamento particolare della matrice. Tale struttura a vescicole si trova anche negli stadi premeiotici dell'oogenesi (Fig. 1).

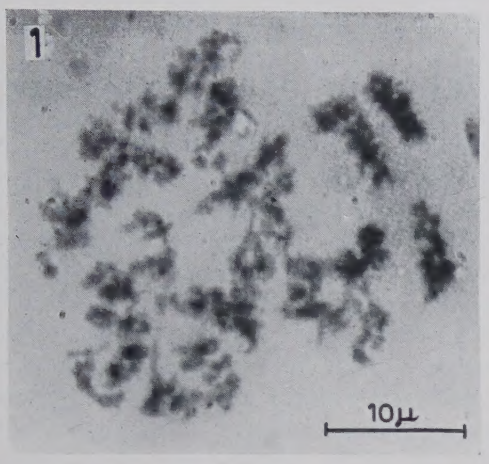

Fig. 1. Nucleo di oocita di *Anacridium aegyptium* L. (Orthoptera) prima della meiosi. I singoli cromosomi sono ben visibili e mostrano la «maior coil». Preparato per schiacciamento, col. FEULGEN.

L'elicoide «maior» dell'anafase precedente, che permane più a lungo, si rilascia soltanto quando si inizia lo stadio di preleptotene, determinando l'andamente sinuoso ed irregolare dei filamenti cromosomici, che poi si raddrizzano al leptotene vero e proprio. Allo zigotene i cromosomi presentano delle irregolarità lungo l'asse, che permangono identiche nel postzigotene (Fig. 2.) Si tratta degli ingrossamenti descritti ancora nel 1917 da WENRICH[1] come cromomeri. Questi supposti cromomeri sono interpretati da RIS[2] come giri di un avvolgimento elicoidale, in questo caso «minor coil», che simulano una struttura cromomerica.

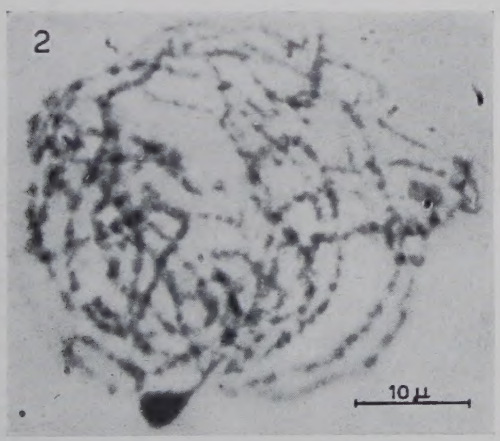

Fig. 2. Nucleo di spermatocita di *A. aegyptium* L. in zigotene. I cromosomi presentano la cosidetta struttura cromomerica dovuta alla «minor coil». Preparato per schiacciamento, col. FEULGEN.

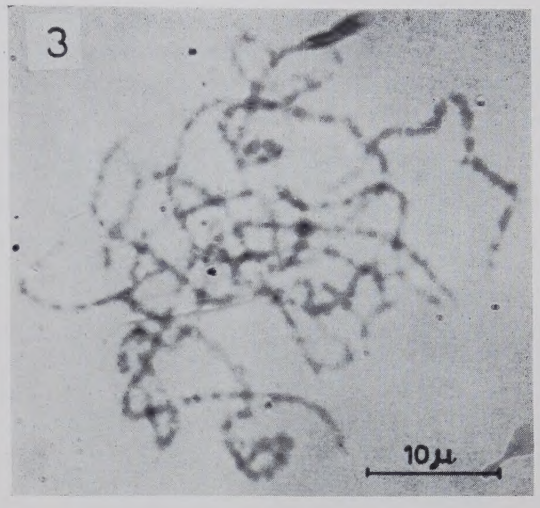

Fig. 3. Nucleo di oocita di *A. aegyptium* in zigotene colla stessa struttura cromosomica degli spermatociti allo stadio corrispondente (fot. 2). Preparato per schiacciamento, col. FEULGEN.

Se si esaminano le trasformazioni cromosomiche nelle prime fasi meiotiche degli oociti, si vede che i nuclei degli oociti fino allo zigotene presentano una struttura simile a quella degli spermatociti degli stadi corrispondenti (Fig. 3).

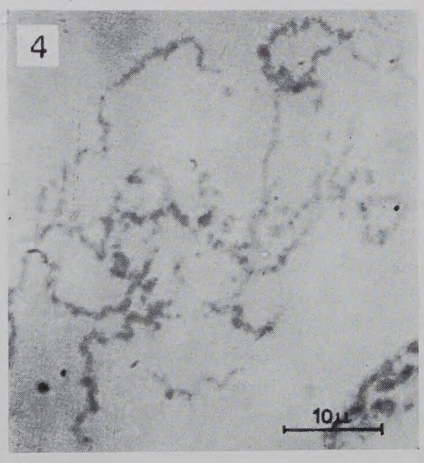

Fig. 4 Nucleo di oocita di *A. aegyptium* L. in postzigotene. La cosidetta struttura cromomerica dello stadio precedente (fot. 3), si risolve in giri più lassi di elicoide. Preparato per schiacciamento, col. con l'ematossilina di HEIDENHAIN.

Nel postzigotene degli oociti, quando inizia il secondo periodo di accrescimento, i cromosomi diventano più lunghi, sottili e sinuosi, lasciando vedere una disposi-

[1] H. S. DAVIS, Bull. Mus. Comp. Zool. Harward Coll. *53*, 60 (1908).

[1] D. H. WENRICH, Bull. Mus. Comp. Zool. Harward Coll. *60*, 58 (1916).

[2] H. RIS, Biol. Bull. *89*, 242 (1945).

zione elicoidale lassa (Fig. 4), fino a che, nei preparati per schiacciamento, anche con la colorazione di Heidenhain, risultano praticamente invisibili.

Nei nuclei isolati, a fresco, col microscopio a contrasto di fase è possibile vedere a questo stadio sottili filamenti spinosi, sparsi in tutto il nucleo, che sono dei cromosomi simili ai cromosomi «lamp-brush» degli oociti degli Anfibi (Fig. 5).

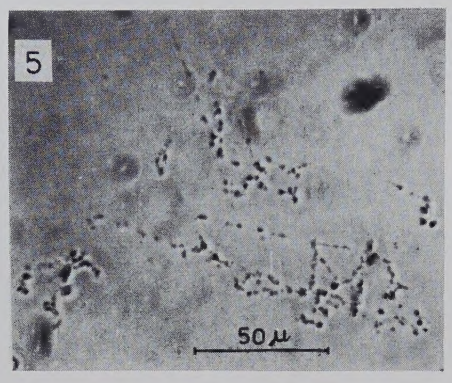

Fig. 5. Cromosomi isolati di un nucleo di oocita in accrescimento. Preparato a fresco, fotografato al microscopio a contrasto di fase.

Le trasformazioni cromosomiche degli oociti in accrescimento perciò si possono interpretare come risultato dello svolgimento della «minor coil» dei cromosomi zigotenici. I cromosomi «lamp-brush» sarebbero costituiti da filamenti cromatidici completamente o quasi estesi.

Queste trasformazioni dimostrano l'esistenza dell'elicoide «minor», che si osserva nelle prime fasi meiotiche degli oociti e che è costante negli spermatociti, come pure in tutte le fasi della mitosi e nell'interfase delle cellule somatiche. Tale conclusione concorda con quanto è stato visto al microscopio elettronico sui cromosomi interfasici isolati (Yasuzumi *et al.*, Polli[1]).

La cosidetta struttura cromomerica dei cromosomi zigotenici e pachitenici degli spermatociti sarebbe perciò dovuta ad avvolgimenti dei cromatidi, come descrive Ris, ma non corrisponderebbe a quella dei cromosomi degli oociti in accrescimento, in cui invece i cromatidi sarebbero completamente svolti. In questi ultimi sono visibili i veri cromomeri costituiti da ingrossamenti o vescicole disposti in fila lungo l'asse del cromatidio e separati l'uno dall'altro da tratti di cromatidio per così dire nudo, come è stato dimostrato negli oociti degli Anfibi da Duryee[2] e da Gall[3].

Nelle fasi meiotiche, che non siano quelle dell'accrescimento oocitario, i giri dell'elicoide «minor» non sono necessariamente distribuiti in modo regolare. Lungo l'asse del cromosoma si trovano delle regioni a giri di avvolgimento più fitti ed altre ad avvolgimenti più radi. Queste strutture corrispondono ai «cromomeri» ampiamente studiati nella loro distribuzione sui cromosomi pachitenici del riso da Lima de Faria[1], il quale del resto dà una definizione morfologica di tali strutture, che non pregiudica affatto l'interpretazione da me data.

Bisogna considerare che la «minor coil» interessa il cromatidio ad un livello strutturale, in cui variazioni nella struttura del cromatidio stesso, dovute ai cromomeri veri fanno sentire la loro influenza sull'avvolgimento stesso. Ciò generalmente non avviene per la «maior coil», poichè essa interessa il cromatidio che già presenta un avvolgimento interno, per cui le caratteristiche morfologiche e fisiologiche dei cromatidi possono far sentire meno i loro effetti; in questo caso è la matrice che assume forse maggiore importanza. Questo risulta per esempio nelle regioni eteropicnotiche degli eterocromosomi (White, Coleman[2]), che sono caratterizzate da un diverso grado di avvolgimento della «maior coil», e da una differente carica di acidi nucleici in confronto degli autosomi.

Rapporti tra cromatidi e cromosomi figli

Abbiamo visto come i cromosomi presentino l'elicoide «minor» costantemente, anche nell'interfase. Questo fatto ed i recenti dati delle ricerche sulla sintesi nucleare dell'ADN permettono di riesaminare le questioni riguardanti i rapporti strutturali dei cromatidi in seguito all'autoriproduzione ed all'accoppiamento.

Le principali conclusioni su questo argomento sono fondate sui risultati delle osservazioni microscopiche sui cromosomi profasici e metafasici della mitosi e della meiosi. Molto spesso però i risultati variano da materiale a materiale e dipendono dai metodi di allestimento dei preparati.

In base ai rapporti tra i cromosomi figli alla metafase gli avvolgimenti cromatidici vengono indicati come «plectonemici», usando la terminologia di Sparrow[3], quando nell'avvolgimento elicoidale «maior» i due cromatidi sono sempre paralleli e non si possono separare senza svolgerli; oppure «paranemici», quando i due cromosomi figli possono essere separati liberamente in direzione laterale. In quest'ultimo caso in proiezione i cromatidi sembrano accavallarsi l'uno sull'altro due volte ad ogni giro di elicoide.

In generale si ammetteva che la «maior coil» della mitosi fosse di tipo plectonemico, mentre nella meiosi si avrebbe un avvolgimento paranemico. Attualmente le opinioni sono piuttosto cambiate: gli avvolgimenti plectonemici sembrano sempre meno probabili.

[1] G. Yasuzumi *et al.*, Chromosoma *4*, 221, 359 (1951). – E. E. Polli, Bioch. Bioph. Acta *10*, 215 (1953).
[2] W. R. Duryee, Ann. N. Y. Acad. Sci. *40*, 920 (1950).
[3] J. G. Gall, J. Morph. *94*, 283 (1954).

[1] A. Lima de Faria, Chromosoma *5*, 1 (1952).
[2] M. J. D. White, J. Genet. *40*, 67 (1940). – L. C. Coleman, Genetics *28*, 2 (1943).
[3] A. H. Sparrow, Canad. J. Res. *20*, 257 (1942).

Nella rivista sintetica sulla struttura elicoidale dei cromosomi di Manton[1] si trova una messa a punto ed un esame critico di questi problemi.

Fig. 6. Schema di cromosoma interfasico od in profase mitotica iniziale, con due cromatidi avvolti secondo l'elicoide minor paranemica.

Qualsiasi opinione si voglia sostenere, per tali questioni non è sufficiente basarsi su singole osservazioni, bensì bisogna prendere in esame tutto il complesso di trasformazioni cromosomiche, dato che le strutture di un determinato stadio non sono che la conseguenza di strutture precedenti.

Nella mitosi l'autoriproduzione cromatidica avverebbe nell'interfase, come dimostrano indirettamente le ricerche citofotometriche sull'ADN di Lison e Pasteels[2], di Swift ed altri[3], cioè avverebbe quando i cromosomi mostrano i residui della «maior coil», le «relic coils», mentre presentano pressochè normale la «minor coil».

Dopo l'autoriproduzione, prima della separazione dei cromatidi figli, che talora si osserva già alla profase, si ha l'individuazione dei cromatidi figli che probabilmente avviene per il fatto che la matrice prima unica per ogni cromosoma si individualizza per i singoli cromatidi. Tale individuazione si può porre già all'interfase.

Nel corso delle trasformazioni cromosomiche durante la profase e la prometafase caratterizzate dalla formazione e contrazione della «maior coil», non si osserva mai nè attorcigliamento nè detorsione dei cromosomi o dei cromatidi. Si deve perciò pensare che all'autoriproduzione si formi una elicoide minor «paranemica» (Fig. 6); alla profase la «maior coil» formerebbe pure un avvolgimento paranemico. Prima della separazione dei cromosomi figli ciascun cromosoma sarebbe perciò formato da due cromatidi con due avvolgimenti, uno incorporato nell'altro, ed entrambi paranemici (Fig. 7). Solo all'anafase i singoli cromosomi sarebbero formati da un singolo cromatidio con doppio avvolgimento (Fig. 8).

Queste conclusioni derivate da un esame puramente morfologico sono particolarmente interessanti se si ricollegano alla probabile struttura cromonemica a livello molecolare: infatti i sali dell'ADN sarebbero disposti in catene elicoidali intorno ad un asse (Watson e Crick[1]). Colla riproduzione cromosomica, attraverso il meccanismo della copie (Muller[2]) o meglio attraverso quello delle copie negative («templates») (Haldane[3]), si originerebbero nuove strutture elicoidali a livello molecolare.

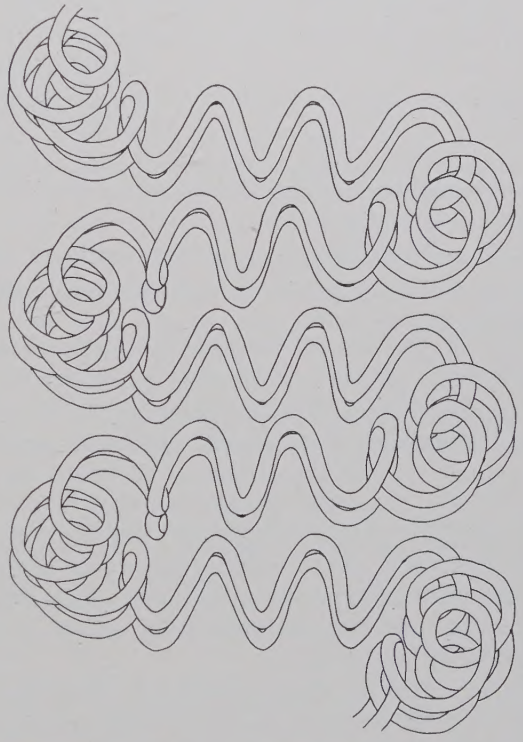

Fig. 7. Schema di cromosoma in prometafase mitotica, con due cromatidi avvolti secondo un'elicoide minor paranemica incorporata in una maior pure paranemica. Questo schema vale anche per gli univalenti alla meta- ed anafase della prima divisione meiotica.

Questo tipo di disposizione, colla formazione di elicoidi a livello strutturale microscopico, determina da

[1] I. Manton, Biol. Rev. *25*, 486 (1950).
[2] L. Lison e J. Pasteels, Arch. Biol. *63*, 1 (1951).
[3] H. Swift, Internat. Rev. Cytol. *2*, 1 (1953).

[1] J. D. Watson e F. H. C. Crick, Nature *171*, 737 (1953).
[2] H. J. Muller, Proc. Roy. London [B] *134*, 1 (1947).
[3] J. B. S. Haldane, *The biochemistry of genetics. Cpt. VIII, Mutation and the problem of gene reproduction* (Allen & Unwin, London 1954).

un punto di vista geometrico, la formazione di avvolgimenti paranemici e perciò la possibilità di individuazione e separazione dei cromosomi figli senza svolgimenti o modificazioni strutturali notevoli.

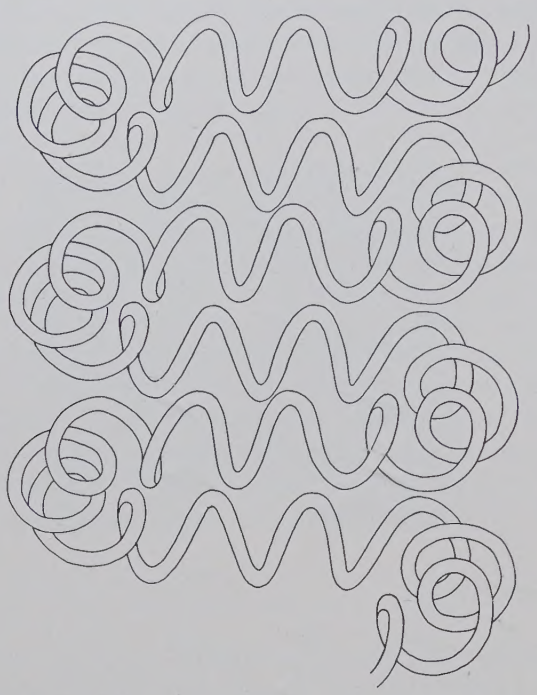

Fig. 8. Schema di cromosoma meta-anafasico mitotico con un solo cromatidio avvolto secondo la «maior» e la «minor coil».

Nella meiosi l'accoppiamento degli omologhi, che si associa all'autoriproduzione, complica ancor più la struttura dei bivalenti.

Secondo Darlington la meiosi è caratterizzata da una «profase precoce» (teoria della «profase precoce»), cioè il nucleo entra in profase prima dell'autoriproduzione cromatidica. I cromosomi leptotenici sarebbero formati da un solo cromatidio e, dal punto di vista strutturale, presentano gli avvolgimenti relitti: allo zigotene gli omologhi si appaiano; soltanto i cromosomi diplotenici sarebbero strutturalmente comparabili con quelli della profase mitotica (cfr. il diagramma delle strutture cromosomiche della mitosi e della meiosi di Darlington[1].)

Poichè all'inizio dello scostamento diplotenico è stato osservato in alcuni casi un attorcigliamento di un omologo con l'altro, Darlington e molti altri citologi parlano di una «relational coil»; viene ammesso perciò che l'accoppiamento avvenga per avvolgimento reciproco dei due cromosomi omologhi. Le «relational coils» a parer mio non presentano, neppure nel materiale in cui si vedono, una regolarità tale da dimostrare un avvolgimento reciproco: negli spermatociti delle cavallette all'inizio dello scostamento diplotenico i «partner» dei bivalenti sono pressochè paralleli.

[1] C. D. Darlington, *Recent advances in cytology* (Churchill, London 1937).

Si deve perciò ammettere un accostamento parallelo degli omologhi, ed essendo questi avvolti secondo l'elicoide «minor», si forma un avvolgimento paranemico. In questo modo si attua nella maniera più semplice un accostamento per una più ampia superficie.

Una chiara dimostrazione che l'autoriproduzione cromatidica avvenga al pachitene, come sostiene Darlington, non è stata data. Le ricerche citofotometriche di Swift[1] sui nuclei meiotici hanno mostrato che la quantità di ADN è quadruplicata già al leptotene iniziale, stadio in cui si dovrebbe perciò porre l'autoriproduzione stessa.

Le osservazione microscopiche non forniscono dati sufficienti per decidere su questo punto; ad es. nei cromosomi «lamp-brush» degli oociti degli Anfibi i singoli cromonemi secondo Duryee[2] sarebbero duplici, secondo Gall[3] semplici.

Anche sul numero effettivo di filamenti cromonemici nei bivalenti le opinioni dei ricercatori sono tutt'altro che concordi.

In alcuni vegetali (*Tradescantia*, *Trillium*, ecc.) i cromosomi metafasici sarebbero tetrapartiti e le tetradi meiotiche ottopartite (Nebel[4]).

Fig. 9. Schema di cromosoma pachitenico, con quattro cromatidi avvolti secondo un'elicoide paranemica minor.

Questa struttura sarebbe dovuta ad una separazione accessoria dei cromatidi («tertiary split»), oltre a quella con la quale si dividono i cromosomi figli. Anche negli animali sono stati riconosciuti più di 2 e 4 cromatidi ed

[1] H. Swift e R. Kleinfeld, Physiol. Zool. *26*, 301 (1953).
[2] W. R. Duryee, Ann. N. Y. Acad. Sci. *50*, 920 (1950).
[3] J. G. Gall, J. Morph. *94*, 283 (1954).
[4] B. R. Nebel, Cold Spring Harbor Symp. quant. Biol. *9*, 7 (1941).

allora si parla di mezzi cromatidi, come per alcune specie di Flagellati (Cleveland[1]), alcune specie di Acridoidei (Makino, Mickey[2]) ed in un genere di Coccidi (Hughes-Schrader[3]).

In ogni caso per definire l'unità cromatidica non ci si può basare sul numero di filamenti che si possono vedere all'esame microscopico, ma bisogna riferirsi a quelli che si possono individuare in base alla trasformazioni e separazioni cromosomiche nelle successive divisioni (Kaufmann[4]).

Di sicuro per la meiosi si può dire che l'autoriproduzione avviene prima del pachitene e, poichè non c'è alcun motivo per non ammettere che l'autoriproduzione non avvenga anche nella meiosi nello stesso modo che nella mitosi, anche i due cromatidi figli di ciascun univalente daranno origine ad una elicoide paranemica minor.

I bivalenti prima del diplotene sarebbero così formati da quattro filamenti tutti presentanti un avvolgimento proprio ed incastrati l'uno con l'altro in modo da formare complessivamente un'elicoide paranemica «minor» (Fig. 9).

Al diplotene ed alla diacinesi si forma e si contrae l'elicoide «maior» e si separano i «partner» di ciascuna coppia; alla metafase ciascun univalente sarebbe formato da due cromatidi con i due avvolgimenti incorporati, entrambi paranemici (Fig. 7). Tale struttura sarebbe propria anche dei cromosomi anafasici della prima divisione meiotica.

Queste conclusioni, anche per i cromosomi meiotici, si fondano sul fatto che non sono state mai dimostrate in maniera evidente modificazioni della struttura cromosomica che possano indicare avvolgimenti reciproci o vere detorsioni tra cromatidi o tra cromosomi omologhi.

Non credo che si possano ammettere nè «relational coils» nè avvolgimenti di tipo plectonemico. – Attualmente, per quanto mi consta, un solo citologo, Matsuura[5], sostiene che le elicoidi meiotiche sono di tipo plectonemico, ciò in relazione alla sua teoria della formazione dei «crossing-over» mediante rottura e saldatura dei cromatidi tra spirali parallele. Però a sostegno di tale interpretazione non sono riportati altri dati se non quelli del citologo giapponese stesso.

Conclusioni

Numerosissime osservazioni sulla struttura dei cromosomi in materiali diversissimi dimostrano concordemente che i cromatidi sono avvolti secondo un'elicoide.

Questa disposizione spaziale ha un'importanza fondamentale nei processi di riproduzione e di trasformazione dei cromosomi («internal mechanics» di Darlington); ma, in molti particolari di importanza notevole, le opinioni dei citologi sono contrastanti.

L'esame comparativo delle trasformazioni cromosomiche, sopratutto nella spermatogenesi e nell'oogenesi, che sono state qui esposte, ci permette di dare un'interpretazione generale sulla struttura cromatidica e sulla disposizione spaziale dei cromatidi nella mitosi e nella meiosi.

I cromatidi sarebbero costantemente avvolti secondo un'elicoide «minor». Lo svolgimento di tale elicoide si osserverebbe soltanto in alcuni casi, ad es. nei nuclei degli oociti in accrescimento. In questi sono visibili i veri cromomeri. I cromomeri comunemente descritti sarebbero invece giri dell'elicoide «minor», distribuiti irregolarmente lungo l'asse del cromosoma in relazione forse alla distribuzione dei veri cromomeri ed alle caratteristiche della matrice.

La costanza dell'avvolgimento elicoidale «minor» ed il fatto che l'autoriproduzione cromatidica avviene nell'interfase, rendono molto verosimile che nei cromosomi a due o quattro cromatidi si formino sempre degli avvolgimenti paranemici. La stessa disposizione spaziale dei cromatidi si avrebbe in seguito all'appaiamento meiotico.

Questo tipo di struttura nella maggior parte dei casi è confermato dalle trasformazioni cromosomiche, che precedono la separazione meta-anafasica dei cromosomi figli, ed è quella che probabilmente meglio concorda coi meccanismi di riproduzione cromosomica.

Summary

Very many observations of the structure of the chromosomes in various materials show, without exception, that the chromatids are coiled like springs.

This spatial arrangements is of fundamental importance in processes of reproduction and changes in chromosomes ("internal mechanics" of Darlington), but in many important particulars the opinions of cytologists differ.

The comparative study of changes in chromosomes, especially during spermatogenesis and oögenesis, which are described here, permits us to give a general interpretation of the chromatid structure and of the spatial distribution of the chromatids in mitosis and meiosis.

The chromatids should be constantly coiled in a "minor" spiral. The uncoiling of such spirals could be observed in some cases only, e.g., in nuclei of growing oöcytes. In this the chromomeres are visible. The chromomeres commonly described should be the coils of the "minor" spiral, distributed irregularly along the axis of the chromosomes, perhaps in relation to the distribution of true chromomeres and to the characteristics of the matrix.

The constancy of the "minor" coil and the fact that chromatid reproduction takes place at the interphase, makes it very likely that in chromosomes with two or four chromatids there is always paranemic coil. The same spatial arrangement of the chromatids should have followed meiotic pairing.

In most cases this type of structure is confirmed by the changes in the chromosomes, which precede the separation of the daughter chromosomes at meta-anaphase, and it is in better agreement with the mechanisms of chromosomes reproduction.

[1] L. R. Cleveland, Trans. Amer. Phil. Soc. *30*, 1 (1949).
[2] S. Makino, J. Fac. Sci. Hokkaido Imp. Univ. [VI] no. 1, 29 (1936). – G. H. Mickey, Amer. Nat. *80*, 446 (1946).
[3] S. Hughes Schrader, Biol. Bull. *78*, 312 (1940).
[4] B. P. Kaufmann, Bot. Rev. *14*, 57 (1948).
[5] H. Matsuura, Chromosoma *3*, 431 (1949).

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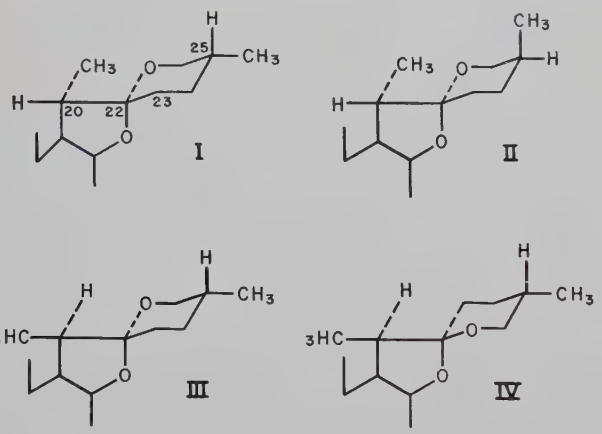
Steroidal Sapogenins. XXX
Stereochemistry of the Side Chain

A number of workers have recently investigated the stereochemistry of the stereoidal sapogenin side chain[1]. The fact that naturally occurring sapogenins have isomerism at C_{25} was established by Scheer, Kostic, and Mosettig, and by James[2]. Work at this laboratory[3] has shown that natural sapogenins have the 20α configuration whereas the unnatural 20-isosapogenins (also called ana-[4], cyclopseudo-[5], and neosapogenins[6]) have the 20β orientation. It has also been recently demonstrated that a true equilibrium is established as a result of heating sapogenins with alcoholic HCl[7], sarsasapogenin and smilagenin each giving a mixture containing approximately 20% of the former and 80% of the latter.



From the foregoing considerations (presented in greater detail in references[8]) the side chain formulation of smilagenin and related 20α, 25D-sapogenins is best represented by I.

Recently, we prepared a number of 20β, 25D- and 20β, 25L-sapogenins[1]. From a comparison of the specific rotations of these sapogenins with their 20α analogues (Table), the author has deduced that formulation II best represents sarsasapogenin and 20α, 25L-sapogenins; formulation III is given to 20-isosmilagenin and related 20β, 25D-sapogenins; and formulation IV best fits 20-isosarsasapogenin and related 20β, 25L-sapogenins.

The basis for the above assignments is the assumption that the highly polar asymmetric center at C_{22} is responsible for the major portion of the observed $[\alpha]_D$ of sapogenins and that the centers at C_{20} and C_{25} have only a minor effect on $[\alpha]_D$. It follows that any major change in the $[\alpha]_D$ of sapogenins must be ascribed to a change at C_{22}.

Let us examine the data which substantiates this assumption. Columns 1 and 2 of Table give the $[\alpha]_D$ of a number of 20α and 20β pairs known to differ at C_{25} in each series[2]. The $[M]_D$ differences for the 20α series are shown in column 3 and are of obvious low magnitude. Column 4 gives the same data for the 20β series and shows a pronounced dextrorotatory shift of large magnitude. We have shown[3] that the C_{25} configurations of sapogenins of the 20β series are identical to their corresponding 20α analogues. Accordingly, we can rule out C_{25} isomerism as a factor in the pronounced dextrorotatory shift observed in the 20β series since we have demonstrated that corresponding C_{25} differences in the 20α series have little effect on rotation. Similarly we can demonstrate that the C_{20} center exerts only a minor effect. Column 6 shows that a change from 20α to 20β in the 25D series has an average effect of about + 45 units. Column 5 shows that the same change from 20α to 20β in the 25L series is of much greater magnitude and of the same order found in column 4 which compares 25L and 25D isomers with the same $C_{20}\beta$ configuration.

One must conclude that the great dextrorotatory shift found in passing to the 20β, 25L series is due to the fact that this group *differs at* C_{22} from its 20β, 25D; 20α, 25D; and 20α, 25L isomers and further that all the other series are *identical at* C_{22}. Furthermore the data supports the view that the highly polar spiroketal C_{22} group is responsible for the major part of the $[\alpha]_D$ values observed with sapogenins. Additional evidence for this view is the fact that whenever the polar spiroketal ring is opened as in the formation of pseudo-, dihydro-, and dihydropseudosapogenins, there is again a pronounced dextro-

[1] I. Scheer, R. B. Kostic, and E. Mosettig, J. Amer. Chem. Soc. *75*, 4871 (1953); *77*, 641 (1955). – V. H. T. James, Chem. and Ind. *1953*, 1388. – M. E. Wall, C. R. Eddy, and S. Serota, J. Amer. Chem. Soc. *76*, 2849 (1954); *77*, 1230 (1955). – R. K. Callow and V. H. T. James, Chem. and Ind. *1954*, 691. – D. H. W. Dickson *et al.*, Chem. and Ind. *1954*, 692. – D. A. H. Taylor, Chem. and Ind. *1954*, 1066. – J. B. Ziegler, W. Rosen, and A. C. Shabica, J. Amer. Chem. Soc. *76*, 3865 (1954); *77*, 1223 (1955). – M. E. Wall, S. Serota, and L. P. Witnauer, J. Amer. Chem. Soc. *77*, 3086 (1955) (in press). – M. E. Wall and H. A. Walens, J. Amer. Chem. Soc. *77* (1955) (in press). – M. E. Wall and S. Serota (MS. in preparation).

[2] V. H. T. James, Chem. and Ind. *1953*, 1388.

[3] M. E. Wall, C. R. Eddy, and S. Serota, J. Amer. Chem. Soc. *76*, 2849 (1954); *77*, 1230 (1955).

[4] R. K. Callow and V. H. T. James, Chem. and Ind. *1954*, 691. – D. H. W. Dickson *et al.*, Chem. and Ind. *1954*, 692.

[5] D. A. H. Taylor, Chem. and Ind. *1954*, 1066.

[6] J. B. Ziegler, W. Rosen, and A. C. Shabica, J. Amer. Chem. Soc. *76*, 3865 (1954); *77*, 1223 (1955).

[7] M. E. Wall, S. Serota, and L. P. Witnauer, J. Amer. Chem. Soc. *77* (1955) (in press).

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Compound	$[\alpha]_D^{0}$ *		$[M_D]$** Differences			
	1 20α	*2* 20β	*3* ΔE_1[a]	*4* ΔE_2[b]	*5* ΔE_3[c]	*6* ΔE_4[d]
Sarsasapogenin 25L	− 75	+ 31[1]	−17	+374	+440	
Smilagenin 25D	− 71	− 59[1]				+ 50
Markogenin 25L	− 70	+ 13[2]	+17	...	+358	
Samogenin 25D	− 74	...				...
Yamogenin 25L	−129	− 15[2]	0	+364	+473	
Diosgenin 25D	−129	−103[2]				+108
		−122[3]				+ 29
3-Desoxysarsasapogenin 25L	− 73	+ 37[2]	− 8	+400	+444	
3-Desoxysmilagenin 25D	− 71	− 63[2]				+ 32
3-Desoxytigogenin 25D	− 69	− 59[2]				+ 40
Tigogenin 25D	− 67	− 67[2]				0

* $[\alpha]_D$ of 20α series determined in chloroform, 20β series in dioxane and converted to chloroform basis by adding (−5) to each observed dioxane value.

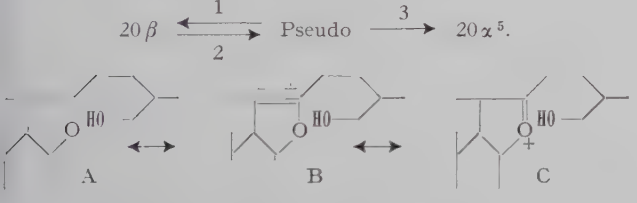
** $M_D = [\alpha]_D \times$ molecular weight/100

[a] $\Delta E_1 = M_D[(20\alpha,25L)-(20\alpha,25D)]$ [c] $\Delta E_3 = M_D[(20\beta,25L)-(20\alpha,25L)]$

[b] $\Delta E_2 = M_D[(20\beta,25L)-(20\beta,25D)]$ [d] $\Delta E_4 = M_D[(20\beta,25D)-(20\alpha,25D)]$

rotatory change in the rotation and the observed $[\alpha]_D$ values are generally near zero[1]. Formulation I has been assigned to smilagenin by several research groups[4] and certainly seems reasonable on the basis of information at hand. It follows from the optical rotation data previously cited that sarsasapogenin is II differing from I only at C_{25}; 20-isosmilagenin is III, differing from I only at C_{20}, and 20-isosarsasapogenin is IV differing from I at C_{20}, C_{22}, and C_{25}. The author proposes that sapogenins of groups I, II, III, IV be called respectively 20α, *22a*, 25D-; 20α, *22a*, 25L-; 20β, *22a*, 25D-; and 20β, *22b*, 25L-sapogenins (G. MUELLER and B. RIEGEL first proposed this nomenclature system).

Finally, let us examine the manner in which the series I–IV could be formed by cyclization of the pseudosapogenins. It is probable that pseudosapogenins exist as resonance stabilized hybrids. In the presence of H^+, cyclization takes place in the sequence:



Assuming cyclization of the planar forms *B* or *C* of pseudosapogenins, there is no longer need to be bound rigidly by the concept of trans ring closures[6]. The nature of the various isomers which are formed seems to depend entirely on *steric effects*.

Considering first the 20α series, it will be noted that models with C_{22} configuration opposite that of I and II indicate that there would be a strong interaction between the C_{21} methyl and the C_{23} methylene groups. Models show much less interaction between the C_{21} methyl and the smaller oxygen atom as shown in I and II so that these forms have less overall energy and are favored. In the case of II, the interaction of the axial C_{27} methyl with a single hydrogen atom has less effect on the overall energy of the molecule than the C_{21}–C_{23} interactions discussed above.

A different situation occurs in the 20β series. In this case the C_{21} methyl is replaced by a much smaller hydrogen atom, and models show no interaction in either of the two C_{22} possibilities. Under these circumstances steric effects at C_{25} might well determine the direction of ring closure so that in each case the more stable equatorial C_{27} methyl is formed. This would require a *cis* closure in the case of III and a *trans* closure in the case of IV. The foregoing rationalization of the cyclization of pseudosapogenins to give the series I–IV is thus in complete accord with the formulations deduced from the optical rotations of these compounds.

There remains for discussion the infrared spectra of I–IV. Originally, we concluded in agreement with R. N. JONES that the large differences between the infrared spectra of I and II were due to differences in C_{22} configuration[1]. The present evidence renders the above hypothesis untenable. Instead the infrared differences between I and II must be ascribed to the equatorial and axial C_{27} methyl group. The markedly different spectre of III[2]. must be ascribed to the strain produced by the 20β configuration, and that of IV both to the 20β and to the difference in C_{22} configuration.

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Eastern Regional Research Laboratory, Eastern Utilization Research Branch, Agricultural Research Service, United States Department of Agriculture Philadelphia 18, Pennsylvania, June 11, 1955.

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[5] M. E. WALL, C. R. EDDY, and S. SEROTA, J. Amer. Chem. Soc. *76*, 2849 (1954); *77*, 1230 (1955). – R. K. CALLOW and V. H. T. JAMES, Chem. and Ind. *1954*, 691. – D. H. W. DICKSON *et al.*, Chem. and Ind. *1954*, 692.

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Zusammenfassung

Die optischen Drehungen einiger 20α, 25D; 20α, 25L; 20β, 25D; und 20β, 25-L-Sapogenine wurden bestimmt. Die ersten drei Serien gaben übereinstimmend linksdrehende Werte, aber die letztere Gruppe erwies sich als rechtsdrehend.

Die Struktur der vier Serien der Sapogenine folgte aus der Analyse dieser Befunde. Der mögliche Mechanismus bei der Entstehung dieser Verbindungen aus Pseudosapogeninen wurde besprochen. Die Autoren gelangen zum Schluss, dass sterische Faktoren an C_{20} und C_{25} die Richtung der Ringschliessung beeinflussen.

Electrolytic Behaviour of Some Carotenoids in Strongly Acid Media

Some time ago, in the course of quite a different investigation[1], we dissolved carotene in isobutylalcohol which was saturated with a solution of 25% sulfuric acid in water, and we electrolysed this solution in an inverted V-tube[2] between two beakers containing 25% sulfuric acid in water into which anode and cathode were immersed. A bluish-green compound migrated towards the cathode and accumulated at the cathodic end of the isobutylalcohol layer. L. Cholnoky, to whom we are indebted for the gift of carotene and for discussions told us, on inspection of the experiment, that his carotene probably contained epoxides and he thought their salts were involved in the electrolytic migration. As a matter of fact, the yellow carotene itself did not migrate.

To identify the green, ionized compound, we prepared authentic β-carotene epoxides with perphthalic acid following Karrer's[3] method and resolved the resulting mixture by chromatography on a $Ca/OH/_2$ column with petrol-ether. Eight different zones were isolated and zones 6. and 7. were identified as Karrer's luteochrome and β-carotene-diepoxide respectively. The maxima of their absorption spectra in carbon disulfide were: 454 and 480 mμ for luteochrome (Karrer's values: 451 and 482 mμ) respectively 470 and 502 mμ for β-carotene-diepoxide (Karrer's values: 470 and 502 mμ). Both compounds were dissolved in the acidified isobutyl alcohol mentioned above. The initially yellow colour of the solutions turned gradually into blueish-green, as described by Karrer, in chloroform containing hydrochloride acid. These green compounds migrated towards the cathode in the above-mentioned inverted V-tube apparatus, applying 90 V to the 3 cm portion of the tube (internal diameter 2 mm) which was filled with the isobutyl alcoholic solution. The current was 5 mA. The green compound did not pass into the aqueous sulfuric acid but accumulated at the cathodic end of the alcoholic phase; it followed the current after reversal of the electrodes.

Other chromatographic fractions of the oxidized carotene behaved similarly, although their colour, which developed after a time in the acidified alcohol, varied between lavender-blue, violet and green. The coloured compound in some cases weakly passed the phase border into the aqueous acid at the cathodic side.

The cathionic behaviour of these carotenoids may be due either to their loosely bound π-electrons or to the formation of oxoniumsalts on the ring-oxygen atoms.

To test these hypotheses, we performed some further experiments. First we tried to electrolyse the chloroform solution of the blue product from carotene and $SbCl_5$ of the Carr-Price reaction, but failed to detect any migration of the blue boundarise between coloured and uncoloured chloroform. This colour reaction is given by carotenoids irrespective of whether they are pure hydrocarbons or contain oxygen. They are held to be dative compounds of the electron-donor carotenoids and the acceptor Lewis-acids ($SbCl_5$, $SbCl_3$, $AlCl_3$, BF_3, etc). In our case the dative compound evidently did not dissociate into positive and negative ions in chloroform solution.

Then we investigated the deep blue compounds which are formed from carotenoids and strong acids. It is well known that the hydrocarbon carotenoids yield these blue compounds with concentrated sulfuric acid, while carotenoids containing oxygen, e.g. violaxanthine, react even with dilute hydrochloric acid. Not much seems to be known, however, about these compounds.

We separated α- and β-carotene chromatographically from each other and examined their reaction with sulfuric acid of varying concentration. They dissolve in concentrated acid to a beautiful peacock-blue solution which however fades through ultramarin to an unsightly violet. Using sulfuric acid diluted with increasing amounts of water, we found that the peacock-blue colour is relatively stable at and beyond 15% water content and the blue colour develops up to about 28% water contents. Using a solution of carotene in benzene and shaking it with sulfuric acid of different concentration, we found that a partition equilibrium is established between the acid and benzene phase. At about 84% H_2SO_4 and above all carotene passes into the acid, while below 72% all of it remains in the benzene or reverts into benzene solution if a stronger acid is diluted with water. At concentrations between 72 and 84% H_2SO_4, the carotene is distributed between the yellow benzene and the blue acid phase reversibly. It should be proved, however, whether the reverted yellow pigment is genuine carotene or a derivate therefrom. We also wish to investigate the partition equilibrium between these phases quantitatively. It is interesting to note that the upper and lower concentration limits of the sulfuric acid at both sides of this partition equilibrium approximate very closely to the composition for $H_2SO_4 \cdot H_2O$ and $H_2SO_4 \cdot 2H_2O$ respectively, although in view of the fact that carotenoids containing oxygen sometimes give the blue colour even with dilute acids, this agreement may prove to be a mere coincidence.

These blue solutions were subjected to an electric field strength of about 1 V/cm in the inverted V-tube apparatus. To stabilize the boundary between coloured solution and the underlying strong sulfuric acid, the former was slightly diluted with water or methanol. To our great surprise these blue derivates of the carotenes migrated to the anode. Their ionic mobilities were of the order of 10^{-4}, which may be regarded as normal in viscous sulfuric acid media. The fractions of the chromatogramm of oxydised carotene mentioned above were treated similarly and this time they too all migrated towards the anode! The cationic or anionic behaviour thus depends upon whether we work in aqueous acid solutions or in acidified isobutyl alcohol (or amyl alcohol). The 6th fraction (luteochrome) dissolved to a grayish-green colour, the 7th fraction (diepoxide) to greenish-blue while the others varied between brown and violet.

[1] F. Körösy and Gy. Székely, Nature *168*, 77 (1951); Magyar Kémiai Folyóirat. *57*, 110 (1951).

[2] F. Körösy and Gy. Székely, Magyar Kémiai Folyóirat. *58*, 174 (1952).

[3] P. Karrer and E. Jucker, Helv. Chim. Acta *28*, 427, 470 (1945).

The anionic behaviour could be explained simply by assuming that sulfuric acid is added to the conjugated system and conveys an acidic character to the carotenoids. However, such a reaction should not involve the change of colour from yellow to blue, nor would it be easy to reconcile this view with the reversibility of the reaction by simple addition of water.

Nothing was known until now about the existence of an electrochemistry of carotenoids. Our experiments revealed not only its existence but also its rather complicated nature. We wish to extend these investigations to a larger number of well defined carotenoids in acids of different strength in different solvents, and we should be glad if specialists in carotenoid chemistry would join in this investigation. We hope to be able to investigate also the magnetic behaviour of the yellow, green and blue solutions.

I wish to express my gratitude to Prof. P. Tuzson for having made me acquainted with carotenoid chromatography and for his valuable advice concerning purification and identification of carotenoids. I am also greatly indebted to Prof. P. Karrer for his valuable and helpful correspondence.

F. Kőrösy

Délibáb u. 28, Budapest, August 3, 1954.

Zusammenfassung

Carotinoide und Carotinoid-Epoxyde bilden mit verschiedenen Säuren und Salzen intensiv blau gefärbte Produkte, deren Natur unbekannt ist. Das elektrolytische Verhalten dieser Stoffe wird untersucht, und es wird festgestellt, dass die Wanderungsrichtung in hohem Mass davon abhängt, ob die Behandlung der Carotinoide mit Säuren in wässeriger oder in wasserfreier alkoholischer Lösung vorgenommen wurde.

The Identity of Vincamajoridine and Akuammine

Akuammine, $C_{22}H_{26}O_4N_2$, the main alkaloid in the seeds of *Picralima Klaineana*[1] and *P. nitida*[2] was recognized as a 5-hydroxy-N-methylindoline as a result of a study of its reactions and of its U.V. and I.R. spectra[2].

Independently, vincamajoridine, recently isolated from *Vinca major* L., was similarly characterized as a 5-hydroxy-N-methylindoline of the formula $C_{22}H_{26}O_4N_2$[3].

The descriptions of the two substances left little doubt in our minds that they were identical and a direct comparison has established that this is indeed the case. The specimen of vincamajoridine, separated chromatographically, was slightly more pure than that of akuammine. Both specimens crystallized from ethanol in short, slender, microscopic needles of identical appearance.

Heated together in capillaries in the same bath vincamajoridine had (uncorr.) m.p. 258–260° (decomp.), and akuammine had m.p. 255° (decomp.); a mixture had m.p. 255–256° (decomp.). The m.p. varied with the rate of heating but a mixture of the two specimens never melted lower than did the akuammine.

The colour reaction with ferric chloride was exhibited identically by the two specimens in respect of colour, intensity, and duration. Vincamajoridine has $[\alpha]_D$-104° ± 4° (pyridine)[1]; we now find that akuammine has $[\alpha]_D^{16}$-105·3° (c, 1·31 in pyridine).

The U.V. absorptions of akuammine[2] and vincamajoridine[1] are the same within experimental error and the complete correspondence of the I.R. spectra is quite remarkable. The curve has already been recorded[1]. Incidentally this agreement in spectra of specimens isolated from different plants, by two groups of workers, and taken on different instruments, shows that even apparently insignificant details are characteristic and signalize real properties of the molecule.

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Résumé

Les auteurs démontrent que la vincamajoridine $C_{22}H_{26}O_4N_2$; F. 258; $(\alpha)_D$-104 ± 4° (py) récemment extraite de la Grande Pervenche (*Vinca major* L.) est identique à l'akuammine antérieurement découverte dans les graines d'une autre Apocynacée *Picralima nitida* (Stapf) T. et H. Durand.

[1] M. M. Janot and J. Le Men, C. r. Acad. Sci. *240*, 909 (9155).
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[3] Laboratory of Galenic Pharmacy, Faculty of Pharmacy, University of Paris.
[4] Dyson Perrins Laboratory, Oxford University.

Oriented Crystallization of Inorganic Salts in Collagen

In an attempt to study in detail the reported "addition compounds" of collagen with alkali[1], the authors observed some interesting phenomena which are reported here. All the experiments described below were performed with kangaroo tail tendon.

A fibre of collagen about 1 mm diameter was kept under tension in 0·25 N sodium hydroxide for about 15 h and was then dried. An x-ray photograph of this specimen is reproduced in Figure 1. In addition to the collagen pattern, the figure exhibits a number of spots on definite layer lines, suggestive of the existence of well crystallized material all oriented parallel to the fibre axis. The spots (excluding those due to collagen) could be indexed on the basis of an orthorhombic unit cell with $a = 5{\cdot}25$ A, b (fibre axis) $= 6{\cdot}45$ A, $c = 10{\cdot}7$ A. On repeating the experiment a second pattern was obtained, which again corresponded to a well crystallized material, oriented with one of its axes along the fibre, but whose unit cell was monoclinic with $a = 20{\cdot}4$ A, b (fibre axis) $= 3{\cdot}5$ A, $c = 10{\cdot}3$ A, $\beta = 106°$. The positions of the spots closest to the direct beam in the first pattern agreed with those reported earlier[1], but those in the second pattern were quite different. The sharpness of the reflections and also the fact that they could be observed right up to $\Theta = 90°$, indicated that they cannot be ascribed to an

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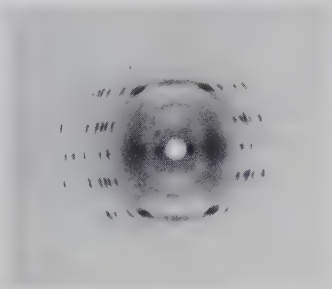
[2] M. F. Millson, R. Robinson, and A. F. Thomas, Exper. *9*, 89 (1953).
[3] M. M. Janot and J. Le Men, C. r. Acad. Sci. *240*, 909 (1955).

[1] G. L. Clark and A. Schaad, Radiology *27*, 339 (1936).

addition compound of collagen. A search of the literature showed that the unit cells of the two patterns agreed closely with those of sodium carbonate monohydrate (Na_2CO_3, H_2O) and sodium sesqui-carbonate (Na_2CO_3, $NaHCO_3$, $2H_2O$) respectively[1]. Thus, it was established that no addition compounds were formed, but that the phenomenon was one of oriented crystallization. Similarly, by keeping collagen in a solution of potassium hydroxide exposed to air, oriented crystallization of $KHCO_3$ has been achieved.



X-ray pattern of collagen in which Na_2CO_3,H_2O has crystallized with its *b*-axis parallel to the fibre axis, taken with a rotation camera 3 cm radius with the fibre axis kept parallel to the axis of rotation of the camera, using CuKα radiation. Photograph reduced by ½ in reproduction.

The specimens containing the crystallized salts are extremely stable, if kept dry, and the x-ray pattern is reproducible even after a month. However, if the specimen is dipped in distilled water for a few seconds and taken out, the salt pattern disappears.

If collagen is kept in freshly prepared sodium hydroxide solution in a full well-stoppered bottle, the above phenomenon was not found, indicating that carbonate produced by contact with air was necessary. So also, if the collagen fibre was kept in a strong solution of sodium carbonate in water, the salt did not enter and crystallize in the collagen. However, if the solution was made alkaline by adding a small amount of sodium hydroxide, then the specimen on drying contained oriented crystals of sodium carbonate. In fact, oriented crystallization of $NaHCO_3$ and Na_2SO_4 has also been achieved by using alkaline solutions of these salts.

It has not yet been possible to explain why the alkali is required. It apparently helps by swelling the collagen and thus enabling the salt to get in. An interesting fact observed with regard to the various salts which crystallized in collagen is that the unit cell dimension along the fibre axis always had a value between 3·2 and 3·75 A or a multiple of this.

A fuller discussion of the experiments will be published in the Proceedings of the Indian Academy of Sciences.

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Department of Physics, University of Madras, Madras 25, March 28, 1955.

Zusammenfassung

Wenn gestreckte Kollagenfasern in einer alkalischen Lösung gewisser anorganischer Salze gelassen und dann getrocknet werden, so kristallisieren die Salze mit einer ihrer Achsen in paralleler Orientierung zur Faserachse. Die Periode parallel dieser Achse liegt zwischen 3,2 und 3,75 Å oder einem Vielfachen dieser Werte.

[1] R.W. G. Wyckoff, *Crystal Structures* (Interscience Publishers 1951), Vol. II, Ch. X, p. 3. – *Structure Reports*, N.V.A. Oosthoek's Uitgevers Mij, Vol. 12, p. 238 (1949).

The Effect of Vitamin B_{12} on the Coenzyme A Content of Normal Strains of *Escherichia coli*[1]

In an earlier investigation, the author observed that the addition of vitamin B_{12} to cultures of "wild" strains of *Escherichia coli* caused an inhibition of the synthesis of free pantothenic acid. It was the author's object in the present report to investigate whether there is any relationship between Vitamin B_{12} and coenzyme A in such strains of *E. coli* as give a positive reaction in the above-mentioned respect. Boxer and his coworkers[2] have previously investigated the relation between vitamin B_{12} and coenzyme A in rat liver cells. They found a definite interrelationship; the coenzyme A content in the liver cells of rats on a vitamin B_{12} deficient diet was considerably higher than the corresponding value of rats receiving additional vitamin B_{12} in the diet.

In his experiments, the author at first isolated from normal human faeces some strains of "wild" *E. coli* and tested the effect of vitamin B_{12} on the pantothenate synthesising ability of these strains. The methods used in these experiments are described in an earlier paper (Jännes[3]). The author only modified the culture medium used in this work in the following way: the earlier medium used contained as source of organic nitrogen seven amino acids: Tryptophane, proline, arginine, histidine, glycine, glutamic acid and asparagine. Instead of these, a solution of vitamin-free casamino acids (Difco) with addition of tryptophane and asparagine was used.

The composition of the medium was as follows: K_2HPO_4, 10 g; KH_2PO_4, 4·5 g; sodium citrate 0·75 g; $(NH_4)_2SO_4$, 1·5 g; $MgSO_4 \cdot 7\ H_2O$, 0·15 g; glucose, 15 g; l-asparagine, 4 g; vitamin-free casamino acids (10% solution), 30 ml; dl-tryptophane, 100 mg; distilled water to make 1000 ml. The pH was adjusted to 7·0.

The procedure was as follows: immediately after the preliminary experiments which were mentioned above, strains whose free pantothenate synthesis was inhibited by added vitamin B_{12} were cultivated on stab agar cultures and kept in a refrigerator. On the following day, the effect of vitamin B_{12} on the coenzyme A content of these strains was examined. It was noted that three of the seven investigated strains showed definite inhibition.

For the assay of coenzyme A, the strain was cultivated in two 2 litre Erlenmeyer bottles, with and without addition of vitamin B_{12}. The same medium as in the preliminary experiment was used. The growth of the bacteria was not in any way affected by the addition of vitamin B_{12}. After 24 h growth the cells were harvested with a Sharpless supercentrifuge and dried in a thin layer over phosphorous pentoxide in a vacuum exsiccator. It was found necessary that the conditions during the drying procedure were absolutely the same for both samples.

Exactly 100 mg of dried bacteria was weighed from each sample and suspended in 3 ml of water. Both suspensions were than boiled in a gaseous flame for exactly the same time.

After centrifugation, the coenzyme A content of the supernatants was determined by the method of Lipmann and Kaplan[4]. The "transacetylase enzyme" was pre-

[1] This work has been aided by grants from Emil Aaltonen Foundation and Sigrid Juselius Stiftelse.

[2] G. F. Boxer, W. H. Ott, and O. E. Shonk, Arch. Biochem. Biophys. *47*, 474 (1953).

[3] L. Jännes, Ann. Acad. Sci. Fenn. Suppl. *61*, 39 (1954); Exper. *10*, 31 (1954).

[4] N. O. Kaplan and F. Lipmann, J. Biol. Chem. *174*, 37 (1948).

pared from pigeon livers. The only noteworthy modification was that frozen liver pieces were pulverised before addition of cold acetone (JÄNNES[1]).

The "transacetylase enzyme" prepared by the author was able to acetylate at most 65 % of sulfanilamide in the reaction mixture when boiling water of yeast or rat liver was used as standard.

The author had no possibility to operate with commercial coenzyme A standards. The following results were obtained in these assays.

Effect of vitamin B_{12} on the pantothenate synthesising ability of *E. coli* strain No. 1.

Vitamin B_{12} per ml of medium	Synthesised pantothenic acid per ml of medium
0·0 γ	0·09 γ
0·3 γ	0·04 γ

The effect of vitamin B_{12} on the pantothenate synthesising ability of this strain was very clear.

Assay of coenzyme A content of cells of this strain gave the following results:

Vitamin B_{12} per ml of medium	Sulfonamide acetylation readings with Beckmann Photometer Wavelength 545 mμ					Coenzyme A in Lipmann units per g of dry bacteria
	Blank test	Maximum acetylation	Readings with boiling water from the bacteria ml 0·05	0·1	0·3	unit
0·0 γ	0·90	0·36	0·71	0·59	0·39	285
0·3 γ			0·71	0·60	0·42	300

In this experiment, the acetylation readings were almost the same and no notable difference in the amounts of coenzyme A in bacteria cultivated with and without vitamin B_{12} could be detected.

A larger addition of vitamin B_{12} had no effect in this respect:

Vitamin B_{12} per ml of medium	Coenzyme A in Lipmann units per g of dry bacteria
0·0 γ	290
1·0 γ	280

The author also isolated strain No. 2 of *E. coli*, which had the following properties:

Vitamin B_{12} per ml of medium	Synthesised pantothenic acid per ml of medium
0·0 γ	0·06 γ
0·3 γ	0·02 γ

[1] L. JÄNNES, Ann. Acad. Sci. Fenn. Suppl. *61*, 39 (1954); Exper. *10*, 31 (1954).

The corresponding values for coenzyme A were:

Vitamin B_{12} per ml of medium	Coenzyme A in Lipmann units per g of dry weight
0·0 γ	305
0·3 γ	310

According to these experiments, the effect of vitamin B_{12} concerns only the synthesis of the amount of pantothenic acid which is liberated by the cells to the medium. It appears probable that the bacterial cells satisfy their own need of pantothenic acid for the synthesis of coenzyme A. The cultivation time in my experiments, however, was short, only 24 h, and data concerning experiments with a longer cultivation time are not available. The growth of the cells was unaffected by the addition of vitamin B_{12}. The observation of MAAS[1] that there exists in *E. coli* an enzyme which is capable of synthesising pantothenic acid from β-alanine and pantoic acid and is not dependent on coenzyme A, is not in disagreement with my results.

SAXENA, GHOTEK, and AGARWALO[2] noted in 1954 that vitamin B_{12} causes a similar effect on the synthesis of thiamine in the metabolism of *E. coli*. These effects have as yet no explanation and therefore deserve further investigation.

J. JÄNNES

Department of Medical Chemistry, University of Helsinki, April 4, 1955.

Zusammenfassung

Auf vitaminfreien Nährböden wurden *Escherichia-Coli*-Stämme kultiviert, die bei Zusatz von Vitamin B_{12} einen deutlichen Rückgang der Abgabe von Pantothensäure in das Nährmedium zeigten.

Die Bestimmung des Coenzym-A-Gehaltes der Bakterien ist mittels der Methode von KAPLAN und LIPMANN durchgeführt worden. Es ergab sich, dass keine grösseren Schwankungen des Coenzym-A-Gehaltes der Bakterien vorkamen, obgleich der Gehalt an freier Pantothensäure in der Nährflüssigkeit deutlich abnahm.

[1] W. K. MAAS, J. Biol. Chem. *198*, 23 (1952).

[2] K. C. SAXENA, S. GHOTEK, and S. C. AGRAWALO, Exper. *10*, 488 (1954).

Ether Soluble Pigments in Interglacial Gyttja

It has been demonstrated that certain circumstances may favour the preservation of plant pigments. Thus TREIBS[1] identified a series of chlorophyll- and haemin-derivates in mesozoic oil-slate, coals etc.; FOX *et al.*[2] showed that carotenoids were present in marine sediments 8000 years old. Recently VALLENTYNE[3] described three chlorophyll degradation products from freshwater sediments aged up to 11,000 years from Canadian lakes.

[1] A. TREIBS, Liebigs Ann. *509*, 103 (1934); *510*, 42 (1934); *517*, 172 (1935); *520*, 144 (1935).

[2] D. L. FOX, D. M. UPDEGRAFF, and D. G. NOVELLI, Arch. Biochem. *5*, 1 (1944).

[3] J. R. VALLENTYNE, Canad. J. Bot. *33* (1955) (in the press).

In the course of recent borings in interglacial freshwater deposits carried out by the Geological Survey of Denmark a gyttja deposit of a remarkably green colour was encountered near Rodebaek in westernmost Jutland. As a preliminary examination revealed that chlorophyll might be responsible for the colour a special investigation of this problem was made.

Origin and Age of the Gyttja. The interglacial deposit Rodebaek I was originally investigated by Jessen and Milthers[1]; the following is a brief summary of their description. The lake deposits were formed in a kettlehole in boulder clay belonging to the penultimate glaciation (Saale-Riss) approximately 125 m across and up to 12 m deep. The general sequence in the deepest part of the basin is,

0 – 0·7 m	Postglacial peat
0·7– 4·7 m	Deposits belonging to the last glacial period (under reinvestigation)
4·7– 5·2 m	Interglacial *Sphagnum* peat
5·2– 6·5 m	Interglacial forest peat
6·5– 7·1 m	Interglacial coarse detritus mud
7·1–10·5 m	Interglacial greenish mud (the stratum under consideration here)
10·5–	Boulder clay.

The deposit was not reached by the last glaciation and is up to the present day essentially undisturbed.

Pollen analysis of the samples for the present investigation show that they belong to the lower part of pollen zone *f* by Jessen and Milthers, which represents the climatic optium of the interglacial period. They consist of a very fine-grained, slightly sandy detritus gyttja. Chemical analysis gave 44·6% loss on ignition, 40·9% Fe_2O_3, 5·0% Al_2O_3, 0·2% Mn, 8·4% silicates, and traces of CaO. The iron has been present to a large degree as ferrous carbonate[2]. Dry matter content was 60·4%, and pH 6·3–6·8. Macroscopical plant remains are very scarce; aquatics identified from this stratum by Jessen and Milthers are: *Batrachium aquatile* L., *Ceratophyllum demersum* L. var. *apiculatum* Cham., *C. submersum* L., *Najas marina* L., *Nuphar luteum* L., *Nymphaea alba* L., and *Potamogeton natans* L. Microscopical examination of unprepared material revealed that the gyttja consists of a structureless organic mass in which only a few pollen grains and diatom shells can be distinguished. In a sample treated with H_2O_2 the following diatoms were the most common: *Cymbella lanceolata* (Ehr.) Van Heurck, *Epithemia turgida* (Ehr.) Kütz., *Gomphonema acuminatum* Ehr., *G. constrictum* Ehr., *Gyrosigma attenuatum* (Kütz.) Rabh., *Navicula placentula* (Ehr.) Grun., *Pinnularia gibba* Ehr., *Synedra ulna* (Nitzsch.) Ehr. together with a number of resting spores of Chrysomonadales and sponge spicules. It is evident that the bulk of the organic matter was formed by a planctonic algal community, the remains of which was not preserved during the fossilization process.

The age in years of the last interglacial period has been estimated by various methods. Zeuner[3] gives the figures 180,000–120,000 years based on Milankovic's solar radiation curve. Its applicability has been questioned *i.a.* by Woldstedt[1]. Flint[2] gives a tentative estimation of 210,000–100,000 years, but in view of recent C^{14} dates these figures are certainly too large. Arrhenius[3], finally, gives the figures 160,000–70,000 years but there is at the present little agreement as to how the material should be interpreted, cp. Wiseman[4]. On the whole, the subject is not quite clear, but an estimated age of about 100,000 years of the Rodebaek gyttja samples cannot be entirely wrong.

Methods. In the field the samples were immediately transferred into a thermos-flask. Parts were also stored in dark glass jars. Later comparison of gyttja stored in this way did not differ in pigment composition from gyttja stored at original temperature. The colour of freshly collected gyttja was dark moss-green. On storage the gyttja became brownish on parts exposed to air.

5 g of non-oxidized sample was shaken in a flask containing glass beads with 5 ml of ethyl ether and filtered through Jena glass-filter 3G3. A clear yellowish extract was obtained which was concentrated *in vacuo* to approximately half volume. Chromatograms were developed on Whatman paper no. 1. The method employed is described by Sironval[5] and is a modification of Bauer's[6] method for chromatography of chlorophylls. The developer consists of benzene : petrolether : acetone 100 : 25 : 20. Chromatograms were run descending in dark 70 cm high glass chambers in an atmosphere of petrolether at a constant temperature of 15°C. After satisfactory separation of the pigments the paper strips were examined partly in daylight, partly under an ultra-violet lamp for determination of fluorescence. Fluorescence spectra were not determined. Visible and fluorescent spots on the chromatograms were separated and transferred to test tubes and a few ml of acetone added. The test tubes were kept in the refrigerator overnight before absorption measurements. These were made in a Beckman quartz spectrophotometer model DU using 10 mm glass cuvettes. Readings were made with 5 mμ intervals between 325 and 800 mμ.

Results. Three types of pigments were found on chromatograms prepared from ether extracts from different levels of the gyttja layer. These are divided according to their colour into green, yellow and red pigments. Figure 1 shows characteristic results of the chromatography. There are five green spots (G_{1-5}), four yellow (Y_{1-4}), and two red (R_{1-2}). In some samples different other ether-soluble pigments were present, but usually these were barely recognizable. The material presented in this report only comprises pigments that have been found repeatedly and being so strong that subsequent reextraction from the chromatograms for measurements of absorption spectra was successful. Minute quantities of non detectable pigments very probably act as contaminants in the detectable pigments; this should be kept in mind in the comparative study of absorption curves. Connecting the starting point and G_3 (see Fig. 1) a faint green longitudinal band with red fluorescence was always seen, but the spots G_1 and G_2 were distinct spots on this background. G_3 was, however, only

[1] K. Jessen and V. Milthers, Danmarks Geologiske Undersögelse, II raekke, *48*, p. 1–379 (1928).

[2] Analysis by K. Skousböll-Hansen, *Geological Survey of Denmark.*

[3] F. Zeuner, *Dating the Past* (Methuen & Co. Ltd., London, 1946).

[1] P. Woldstedt, *Das Eiszeitalter I*, 2. Aufl. (Ferdinand Enke Verlag, Stuttgart, 1954).

[2] R. F. Flint, *Glacial Geology and the Pleistocene Epoch* (New York: John Wiley & Sons Inc., London: Chapman & Hall, Ltd. 1947).

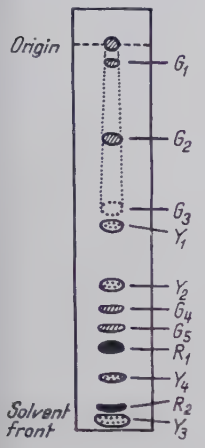
[3] G. Arrhenius, Rep. Swedish Deep-Sea Exped. 1947–48 *5*, fasc. 1, 187 (1952).

[4] J. D. H. Wiseman, Proc. roy. Soc. [A] *222*, 296 (1954).

[5] C. Sironval, Bull. Soc. roy. Bot. Belg. *85*, 285 (1953).

[6] L. Bauer, Naturwissenschaften *39*, 88 (1952).

recognized as a separate spot on its strong fluorescence. All other spots by the chromatograms were well separated and were recognized by colour and fluorescence (R_1 showed no fluorescence). R_f values and relative strength of the pigments in visible and ultra-violet radiation will be found in Figure 1.



	R_f	Visual light		Ultra-violet radiation	
		Colour	Strength	Fluorescence	Strength
G_1	0·06	green	+++	grey-red	++
G_2	0·26	green	++	red	++
G_3	0·44	—	—	red	+++
Y_1	0·49	yellow	++	grey-red	+
Y_2	0·64	yellow	++	grey-red	+
G_4	0·70	green	+	red	+++
G_5	0·75	green	+	red	+++
R_1	0·81	pink	++	—	—
Y_4	0·88	yellow	+	grey-red	++
R_2	0·96	pink	++	grey-red	+
Y_3	0·99	yellow-orange	++++	grey-red	+++

Fig. 1.–Chromatogram of ether-soluble pigments from gyttja.

Green pigments. The five green pigments isolated seem to be closely related. They all have their blue maxima in the 410–420 mμ region and their red maxima between 670 and 680 mμ (Fig. 2 and 3). However, the ratio max. blue/max. red differs considerably from compound to compound: $G_1 = 4{\cdot}3$, $G_2 = 3{\cdot}7$, $G_3 = 4{\cdot}0$, $G_4 = 2{\cdot}7$ and $G_5 = 2{\cdot}3$. Although it seems difficult to identify the pigments in comparing data from known pigments isolated from different sources, cp. RABINOWITCH[1], it could be stated that the five green compounds are derivates or degradation products of chlorophylls originating mainly from phytoplancton which populated the interglacial Rodebaek lake about a hundred thousand years ago. It is astonishing that the pigments have been so well preserved as the samples examined under the microscope were almost completely without cell structure. But as the cells from the photosynthetic layer of the water settle to form the bottom sediment both the amount of light and oxygen decrease considerably, and also the temperature will be low and constant in the bottom sediment. Conditions for autocatabolism have thus been unfavourable, and degradation of the pigments by anaerobic microorganisms apparently does not take place.

The green pigments, except G_1, all showed a bright red fluorescence of high intensity when the chromatograms were examined in ultra-violet radiation. G_1 showed a type of fluorescence which is also characteristic for chlorophyll *b* when examined on paper after chromatographic separation. Only the periphery of the spot appears bright red, whereas the centre could be described as being red with a heavy grey shade covering the colour.

Yellow pigments. The absorption curves of the yellow pigments Y_1, Y_2 and Y_4 indicate that fucoxanthol and luteol or related compounds are present (Fig. 4). The bulk absorption region of these compounds is between 400 and 500 mμ with three characteristic peaks, the position of which depends on the solvent used, cp. KARRER and JUCKER[2]. The fourth yellow pigment Y_3, on the chromatogram situated near the solvent front, seems to be a mixture of compounds mainly consisting of

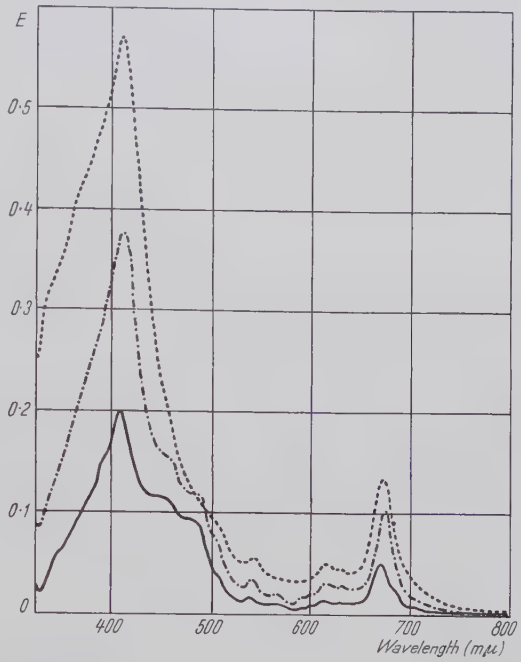


Fig. 2.–Green pigments (1).
Absorption spectra in acetone. ····· G_1; —·—·— G_2; ——— G_3.

β-carotene. The further separation of these pigments by means of paper chromatography was not successful.

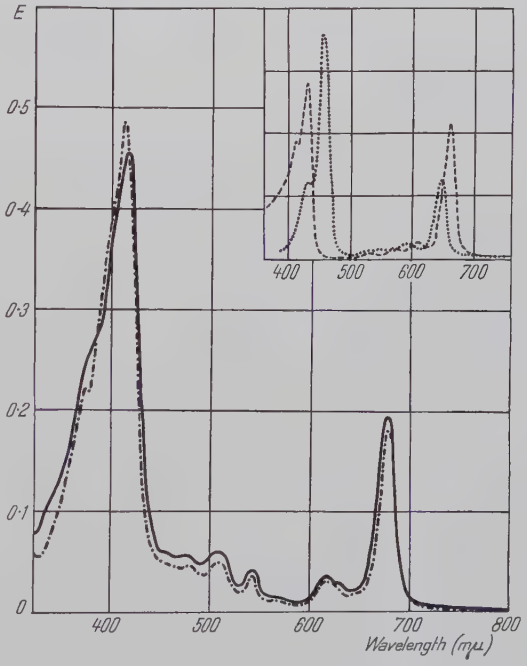


Fig. 3.–Green pigments (2).
Absorption spectra in acetone. —·— G_4; —— G_5. Inserted absorption spectra in acetone of chlorophyll *a* (———) and chlorophyll *b* (·····).

Red pigments. The spots R_1 and R_2 in normal light appear reddish or pink, and the presence of phycobilins was therefore suspected. Phycobilin pigments, however,

[1] E. RABINOWITCH, *Photosynthesis*, Vol. II/1 (Interscience, New York, 1951).
[2] P. KARRER and E. JUCKER, *Carotinoide* (Birkhäuser Verlag Basel, 1948).

have their maximal absorption in the 600 mμ region whereas the *R*-pigments do not show any absorption at

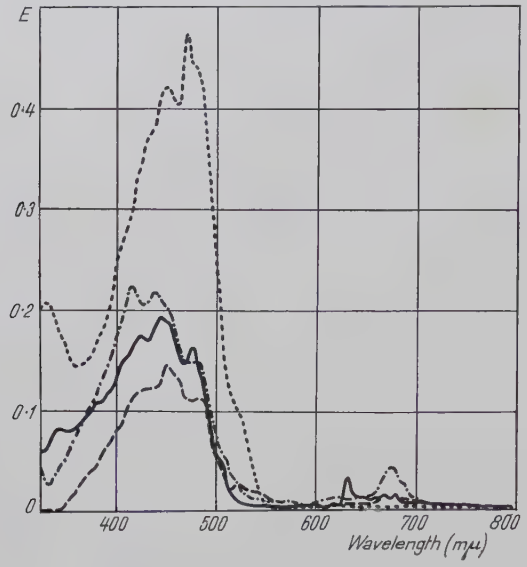


Fig. 4.–Yellow pigments.
Absorption spectra in acetone. ——— Y_1; —·— Y_2; ····· Y_3; —— Y_4.

all at this wavelength (Fig. 5). Therefore Phycobilins were excluded. Carotenoids may, however, also have red

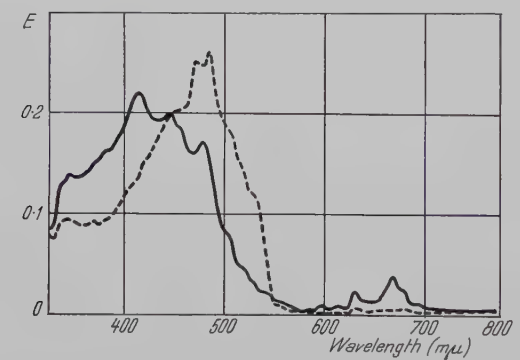


Fig. 5.–Red pigments.
Absorption spectra in acetone. —— R_1; ——— R_2.

colours, and the absorption curve of R_1 make it probable that the compound belongs to this group and also is related to luteol of fucoxanthol. The nature of R_2 is uncertain; the major absorption of this pigment lies about 30 mμ further toward longer wavelengths with two maxima, 470 and 485 mμ.

A survey of main and accessory absorption maxima for the compounds described is given in the Table. The final identification of the different pigments found in the gyttja would necessitate the isolation of the compounds in a solid state which is only possible by large scale separation on absorption columns.

Absorption maxima of pigments in acetone

	Main peaks mμ	Accessory peaks mμ
Green pigments		
G_1	410, 672·5	540, 615
G_2	412·5, 675	540, 615
G_3	410, 670	540, 615
G_4	415, 680	375, 510, 545, 620
G_5	417·5, 677·5	505, 542·5, 620
Yellow pigments		
Y_1	450	430, 485
Y_2	417·5, 440	480, 675
Y_3	330, 450, 470	
Y_4	445	345, 425, 477·5
Red pigments		
R_1	410	345, 445, 477·5
R_2	470, 485	345

S. Th. Andersen and K. Gundersen

Geological Survey of Denmark, Charlottenlund, and Institute of Plant Physiology, University of Copenhagen, June 25, 1955.

Zusammenfassung

Etwa 100000 Jahre alte, interglaziale Gyttja aus Rodebäk im westlichen Dänemark (Riss–Würm-Interglazialzeit) wurde mit Äther extrahiert und die Extrakte mittels Papierchromatographie getrennt und untersucht. Es wurden grüne, gelbe und rote Pigmente gefunden. Aus den Absorptionsspektren und der Fluoreszenz dieser Stoffe ergibt sich, dass die grünen Pigmente Chlorophyllderivate, die gelben (sowie wenigstens eines der roten) Karotinoide sind. Die Gyttja enthielt sehr wenige bestimmbare Pflanzenreste, hauptsächlich nur Pollen und Diatomeen. Die Farbstoffe müssen von lebenden Pflanzen im interglazialen See, in erster Linie Algen, herrühren, und haben infolge Lichtabschluss und niedrigem Redoxpotential sowie niedriger Temperatur ausserordentlich gute Aufbewahrungsbedingungen gefunden; normalerweise werden solche Stoffe schnell abgebaut.

The Action of Hydrogen Peroxide on Amino Acids in Presence of Iron Salts and its Bearing on Photolysis of Amino Acids

Earlier studies[1] on the action of Fenton's reagent on amino acids showed that they are deaminated and converted to aldehydes and corresponding carboxylic acids. Recently Johnson *et al.*[2] have shown that α-keto acids are formed by the action of Fenton's reagent on α-amino acids. They have further pointed out that certain enzymatic processes can be simulated by reactions involving free radicals *in vitro*. Therefore the importance of the study of the action of Fenton's reagent on the amino acids is obvious.

We have observed during our experiments that the amino acids undergo a series of complicated changes by the action of hydrogen peroxide in presence of iron salts. A typical experiment carried out to study the action of Fenton's reagent was as follows.

To 0·2 cm³ of 0·1 *M* solution of amino acid was added dropwise 0·2 cm³ of 0·1 *M* ferrous sulphate solution and the volume was made up to 1·8 cm³ with distilled water. To this 0·2 cm³ of H_2O_2 (0·1 *M*) was added and the tube shaken well for about 3 min. Controls were also kept with H_2O_2 alone and also with ferrous sulphate in absence of hydrogen peroxide. After vigorous shaking, the tubes

[1] H. D. Dakin, J. Biol. Chem. *1*, 171 (1905). – C. Neuberg, Biochem. Z. *20*, 531 (1909). – H. Wieland and W. Franke, Ann. Chem. *457*, 1 (1927).

[2] G. R. A. Johnson, G. Scholes, and J. Weiss, Science *114*, 412 (1951).

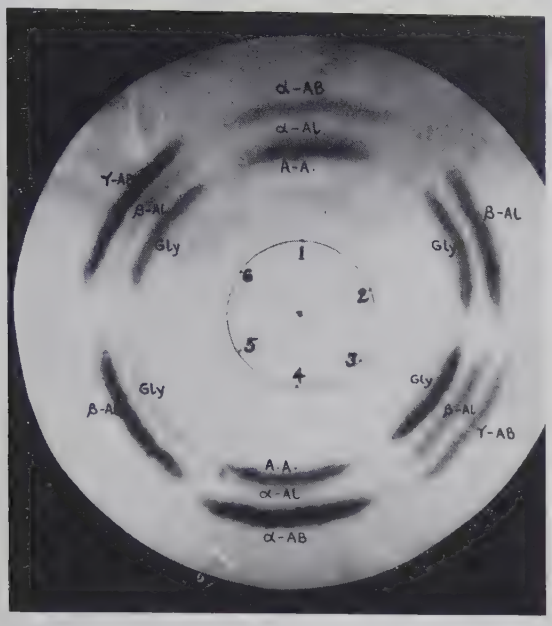


Fig. 1.–Chromatogram showing the action of hydrogen peroxide on amino acids in presence of ferrous and ferric salts (Temperature 24–25°C).
(1) α-aminobutyric acid + H_2O_2 + $FeSO_4$
(2) β-alanine + H_2O_2 + $FeSO_4$
(3) γ-aminobutyric acid + H_2O_2 + $FeSO_4$
(4) α-aminobutyric acid + H_2O_2 + $FeCl_3$
(5) β-alanine + H_2O_2 + $FeCl_3$
(6) γ-aminobutyric acid + H_2O_2 + $FeCl_3$
(α-AB = α-aminobutyric acid, β-Al = β-Alanine, α-Al = α-Alanine, γ-AB = γ-aminobutyric acid, A.A = Aspartic acid, Gly = glycine)

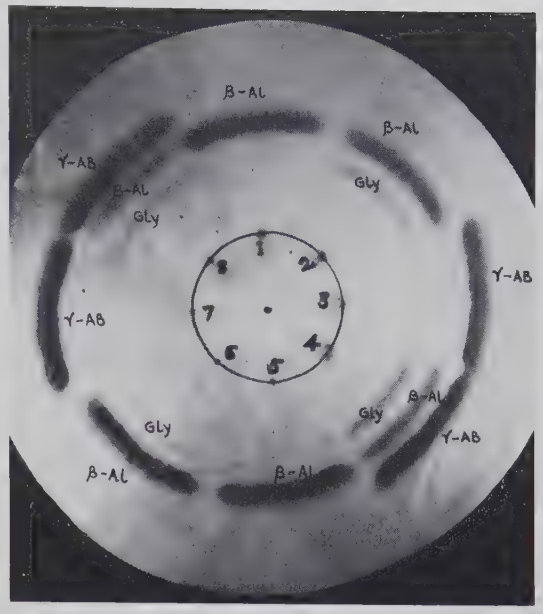


Fig. 2.–Chromatogram showing the photodegradation of amino acids.
(1) β-alanine + $FeCl_3$ in dark
(2) β-alanine + $FeCl_3$ in sunlight
(3) γ-aminobutyric acid + $FeCl_3$ in dark
(4) γ-aminobutyric acid + $FeCl_3$ in sunlight
(5) β-alanine + $FeSO_4$ in dark
(6) β-alanine + $FeSO_4$ in sunlight
(7) γ-aminobutyric acid + $FeSO_4$ in dark
(8) γ-aminobutyric acid + $FeSO_4$ in sunlight
(β-Al = β-alanine, γ-AB = γ-aminobutyric acid, Gly = glycine).

were kept for about 10–15 min at room temperature (24–25°C) and the reaction mixtures were examined for the presence of amino acids by two-dimensional paper chromatography (solvents: butanol-acetic acid-water, 40:10:50 followed by either water-saturated phenol or pyridine-water, 80:20) and also by the circular paper chromatographic technique. The interference by iron salts was overcome by exposing the spot to liquor-ammonia when the iron complex was rendered more mobile and moved with the solvent front (solvent: butanol-acetic acid-water). The chromatogram showing the degradation of amino acids by H_2O_2 in presence of ferrous and ferric salts is given in Figure 1. The amino acids studied were α- and β-alanine. α-amino butyric acid, γ-amino butyric acid, aspartic and glutamic acids and serine. The products formed were the same irrespective of whether ferrous or ferric salts were used. β-Alanine and serine gave glycine; γ-amino butyric acid in turn gave β-alanine and glycine, Glutamic acid gave rise to only aspartic acid, whereas in the case of α-alanine and aspartic acid, ninhydrin-positive products could not be detected. From α-amino butyric acid, the formation of aspartic acid and α-alanine was observed. The amino acids were stable in presence of H_2O_2 alone at room temperature.

It is well-known that when ferrous and ferric salts are added to H_2O_2, it goes through a series of reactions giving rise to free radicals[1]. We have found that the amino acids are degraded by FENTON's reagent only when the molar concentration of ferrous salts is equal to or less than that of H_2O_2. The absence of reaction when the molar concentration of ferrous ions was greater than that of H_2O_2 may be explained by the fact that in this case the regeneration of free radicals by chain mechanism is restricted[1].

The reactions reported here have a striking resemblance to the photolysis of amino acids in presence of TiO_2[2] and to the action of H_2O_2 at 100°C[3]. Considering the close similarity between the action of FENTON's reagent and the photosensitized action of TiO_2 on amino acids, it can be suggested that during the photolysis of amino acids by sunlight in presence of TiO_2, H atoms and OH radicals are generated by dissociation of the water molecule and that the OH radicals are responsible for the oxidative reactions reported earlier[2].

While studying the photolysis of amino acids, it was observed by us that in addition to metal oxides like TiO_2 and ZnO, ferrous sulphate and ferric chloride also acted as efficient photosensitizers and gave the same type of degradative reactions with some of the amino acids, namely, γ-amino butyric acid and β-alanine (Figure 2).

Further studies on the role of these photosensitizers in the oxidative degradation of amino acids are in progress.

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C. S. VAIDYANATHAN, and K. V. GIRI

Department of Biochemistry, Indian Institute of Science, Bangalore 3, India, January 17, 1955.

[1] F. HABER and J. WEISS, Proc. Roy. Soc. [*A*] *147*, 332 (1934). – W. G. BARB, J. H. BAXENDALE, P. GEORGE, and K. R. HARGRAVE, Trans. Faraday Soc. *47*, 462, 591 (1951).

[1] W. G. BARB, J. H. BAXENDALE, P. GEORGE, and K. R. HARGRAVE, Trans. Faraday Soc. *47*, 462, 591 (1951).

[2] K. V. GIRI, G. D. KALYANKAR, and C. S. VAIDYANATHAN, Naturwissenschaften *40*, 440 (1953); *41*, 88 (1954).

[3] Y. MATSUO, Nature *171*, 1021 (1953).

Zusammenfassung

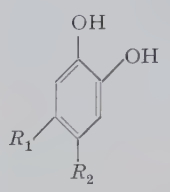
Es wurde der Abbau von Aminosäuren durch Wasserstoffsuperoxyd in Gegenwart von Eisensalzen untersucht. Papierchromatographische Analyse zeigte, dass dabei aus γ-Aminobuttersäure β-Alanin und Glycin, aus β-Alanin und Serin Glycin entsteht. Die Bildung von α-Alanin und Asparaginsäure aus α-Aminobuttersäure und von Asparaginsäure aus Glutaminsäure konnte ebenfalls festgestellt werden. Diese Resultate werden in Zusammenhang gebracht mit dem Mechanismus der Photolyse von Aminosäuren in Gegenwart von TiO_2.

Activité staphylostatique de quelques diphénols, naphtols et chalcones nouveaux

Au cours de nos recherches sur les mécanismes de l'action antibactérienne des substances organiques en relation avec leur structure moléculaire[1], nous avons été amenés à examiner l'activité staphylostatique de nombreux composés nouveaux appartenant à trois familles chimiques: diphénols, naphtols et chalcones. En effet, dans ces trois groupes, on avait déjà trouvé des dérivés fortement bactériostatiques[2].

La méthode expérimentale utilisée dans les présentes recherches consiste à ensemencer des tubes de bouillon nutritif contenant la substance à examiner, avec une goutte d'une culture de 18 h de *Micrococcus pyogenes* var. *aureus* diluée au $1/10^6$. La pousse microbienne est suivie visuellement tous les jours pendant une durée de 3 ou 4 jours. Dans ces conditions, une étude effectuée sur 138 composés appartenant aux trois groupes chimiques sus-indiqués[3] a permis de déceler chez quelques-uns d'entre eux une activité staphylostatique importante (c'est-à-dire à des concentrations comprises entre 10^{-5} et 10^{-6}).

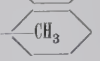
Voici quelques-uns des résultats les plus significatifs ainsi obtenus (voir tableau I).

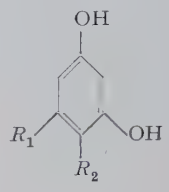


I; $R_1 = H$, $R_2 = -C(CH_3)_3$

II; $R_1 = Cl$, $R_2 = -C(CH_3)_3$

III; $R_1 = H$, $R_2 =$

IV; $R_1 = H$, $R_2 =$ 

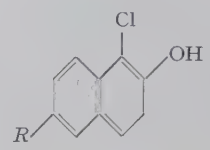


V; $R_1 = H$, $R_2 = tert.\text{-}C_5H_{11}$

VI; $R_1 = H$, $R_2 =$

VII; $R_1 = Cl$, $R_2 =$

(Voir tableau II).



VIII; $R = -C(CH_3)_3$

IX; $R = tert.\text{-}C_5H_{11}$

X; $R =$

(Voir tableau III).

En résumé, il est montré que certains dérivés substitués du résorcinol et du β-naphtol, ainsi qu'une chalcone dérivée du thiophène, la *p*-chlorobenzal(5-nitro-2-acétothiénone), possèdent une activité staphylostatique élevée. Cette activité dépend, dans une grande mesure, de la structure moléculaire. Signalons par ailleurs qu'aucune des substances étudiées n'a montré d'activité bactériostatique contre *Escherichia coli*, en bouillon nutritif, et à la concentration de 10 γ/ml; et dans quelques cas, nous avons vérifié qu'il en est de même pour la concentration de 100 γ/ml.

M. Welsch, N. P. Buu-Hoï et F. Binon

Institut de Bactériologie de l'Université de Liège et Institut du Radium de l'Université de Paris, le 19 avril 1955.

[1] Voir N. P. Buu-Hoï et M. Welsch, Exper. *10*, 248 (1954). – A. Lacassagne, N. P. Buu-Hoï, F. Zajdela et N. D. Xuong, C. r. Acad. Sci. *231*, 89 (1950).

[2] Voir W. A. Sexton, *Chemical Constitution and Biological Activity* (E. and F. N. Spon Ltd., London 1949), p. 249; pour les chalcones, voir E. Schraufstätter et S. Deutsch, Z. Naturforsch. *36*, 430 (1948).

[3] La plupart des composés étudiés ont déjà été décrits chimiquement dans les publications suivantes: N. P. Buu-Hoï, H. Le Bihan, F. Binon et P. Rayet, J. Org. Chem. *15*, 1060 (1950). – N. P. Buu-Hoï, H. Le Bihan et F. Binon, J. Org. Chem. *16*, 185 (1951). – N. P. Buu-Hoï, H. Le Bihan et N. D. Xuong, J. Org. Chem. *16*, 987 (1951). – N. P. Buu-Hoï, H. Le Bihan, F. Binon et P. Maleyran, J. Org. Chem. *18*, 4 (1953).

Tableau I. Groupe des diphénols

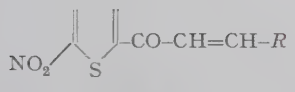
Substance	Formule	Concentration staphylostatique minimale en γ/ml
4-tertiobutylpyrocatéchine	I	retard de croissance à 10
5-chloro-4-tertiobutylpyrocatéchine	II	retard de croissance à 10
4-cyclohexylpyrocatéchine	III	retard de croissance à 10
4-(1-méthyl-1-cyclohexyl)-pyrocatéchine	IV	retard de croissance à 10
4-cyclohexylgaïacole		retard de croissance à 10
4-(1-méthyl-1-cyclohexyl)-gaïacole		retard de croissance à 10
chloro-4-tertiobutylgaïacole		5
bromo-4-tertiobutylgaïacole		retard à 10
4-tertioamylrésorcinol	V	5
4-(1-méthyl-1-cyclohexyl)-résorcinol	VI	1,25
5-chloro-4-(1-méthyl-1-cyclohexyl)-résorcinol	VII	1,25
2,7-dicyclohexylfluorescéine		retard à 10
2,7-dicyclohexyléosine		retard à 10

Tableau II. Groupe des naphtols

Substance	Formule	Concentration staphylostatique minimale en γ/ml
6-bromo-2-naphtol		retard à 10
6-tertioamyl-2-naphtol		10
1-chloro-6-tertiobutyl-2-naphtol	VIII	5
1-chloro-6-tertioamyl-2-naphtol	IX	5
1-bromo-6-tertioamyl-2-naphtol		5
1-bromo-6-tertiobutyl-2-naphtol		10
6-(1-méthyl-1-cyclohexyl)-2-naphtol		10
6-(1-éthyl-1-cyclohexyl)-2-naphtol		10
1-chloro-6-(1-méthyl-1-cyclohexyl)-2-naphtol	X	2,5
1-bromo-6-(1-méthyl-1-cyclohexyl)-2-naphtol		10
1-chloro-6-tertiobutyl-2-méthoxy-naphthalène		> 10
1-chloro-6-(1-méthyl-1-cyclohexyl)-2-méthoxynaphthalène		> 10

Tableau III. Groupe des chalcones

Substance	Formule	Concentration staphylostatique minimale en γ/ml
furylidène-(5-nitro-2-acétothiénone)	XI	retard à 10
furylidène-(5-bromo-2-acétothiénone)		retard à 10
benzal-(5-nitro-2-acétothiénone)	XII	10 (retard à 1)
o-méthoxybenzal-(5-nitro-2-acétothiénone)	XIII	10
p-chlorobenzal-(5-nitro-2-acétothiénone)	XIV	1
p-diméthylaminobenzal-(5-nitro-2-acétothiénone)	XV	retard à 10
2-thénylidène-parafluoroacétophénone		retard à 10
2-thénylidène-(5-éthyl-2-acétothiénone)		retard à 10
5-méthyl-2-thénylidène-(5-éthyl-2-acétothiénone)		retard à 10
vératrylidène-parachloroacétophénone		retard à 10



XI; $R =$

XII; $R = -C_6H_5$

XIII; $R = -C_6H_4(OCH_3)_{(o)}$

XIV; $R = -C_6H_4Cl_{(p)}$

XV; $R = -C_6H_4\text{-}N(CH_3)_{2(p)}$

Summary

Some substituted derivatives of resorcinol and β-naphthol, and a chalkone derived from thiophene, namely *p-chlorobenzal(5-nitro-2-acetothienone)*, are shown to possess high bacteriostatic activity towards *Micrococcus pyogenes* var. *aureus*; no such activity was found towards *Escherichia coli*, even at higher concentrations.

The Distribution of Mitochondria in Sea Urchin Embryos[1]

It has been reported[2] that in developmental stages of *Psammechinus miliaris*, observed after staining with Nile Blue sulfate, mitochondria are not present in large numbers until the mesenchyme blastula and young gastrula stages, at which time a gradient of the organelles appears, decreasing from the animal to the vegetal pole. The presence of such a gradient was established by counting relative numbers of mitochondria along the animal-vegetal axis. Localized centers of mitochondrial production were observed later in development, suggesting to the authors that the differentiation of an organ rudiment is correlated with the appearance of mitochondrial populations in it. Changes in mitochondrial distribution in embryos treated with lithium or iodosobenzoic acid were interpreted in terms of a double gradient of metabolic types, the animal-vegetal gradient favoring mitochondrial production, the opposite one inhibiting it.

The present studies concern mitochondrial distribution in normal developmental stages of two sea urchins, *Lytechinus pictus* and *Strongylocentrotus purpuratus*. Observations on eggs and embryos with phase contrast, or after vital staining, or after fixation and staining according to methods recommended for the visualization of mitochondria, have led to somewhat different conclusions from those cited above, for this material.

Method. The conditions of observation described by Gustafson and Lenicque[1], using vital dyes, were duplicated insofar as possible. After staining with Nile Blue sulfate[2] or Janus grêen[3] (dye concentrations of 0·05–0·1 %), eggs or embryos[4] were flattened under a

[1] Research supported by the National Cancer Institute, National Institutes of Health, United States Public Health Service.

[2] T. Gustafson and P. Lenicque, Exp. Cell Res. *3*, 251 (1952).

[1] T. Gustafson and P. Lenicque, Exp. Cell Res. *3*, 251 (1952.

[2] Grübler (Leipzig) stain.

[3] Nat. Aniline & Chem. Co., New York: Cert. No. Nj-1.

[4] Cultures maintained at 17°C.

vaseline-ringed cover glass. Specimens in the desired orientation were studied under oil immersion[1]. In attempting counts along the animal-vegetal axis, an eye piece reticule of 10 × 10 squares was employed. With later stages (mesenchyme blastula, gastrula), counts were tried on enlarged photographs of both vitally stained and fixed material.

Preliminary trials with several fixatives and stains indicated that the method recommended by Harman[2] for the visualization of mitochondria produced the best results. Large numbers of eggs or embryos can be fixed (Altmann's fluid or osmium tetroxide, 2%) and flattened between cover slips according to the procedure described by Tyler[3]; the specimens were stained with fast green[4] at 60°C and counterstained with safranin[5], cleared and mounted in balsam or Clarite.

Observations on homogenates of eggs or embryos, prepared in 0·78 or 1·1 molar sucrose, made up in phosphate buffer, 0·005 molar, pH 7·5, were made with an oil immersion dark M phase objective.

Results. Phase contrast study of homogenates of *L. pictus* and *S. purpuratus* revealed that, contrary to the report of Gustafson and Lenicque[6] for *P. miliaris*, there are large numbers of cytoplasmic inclusions corresponding to mitochondria in size, shape and structure, in unfertilized eggs and early stages. In homogenates of mesenchyme blastulae and young gastrulae there is an increase in rodshaped mitochondria, spherical or ovoid forms predominating in the earlier stages. Although Gustafson and Lenicque[6] give no size range for the mitochondria observed in *P. miliaris*, it is possible that the mitochondrial precursors which they describe, in early stages, as "very small blue units", correspond to smaller mitochondria (0·5–1·0 micra). Particles in this size range have been observed by Harvey[7], in *Arbacia punctulata*, and in the present studies, in the "clear quarters" of eggs fragmented by centrifugation. It has recently been suggested[8], after a study of electron micrographs of ultra-thin sectioned centrifuged eggs of *Arbacia*, that these "fine granules" may be smaller mitochondria.

Examination of early blastula wall cells, which develop ciliated borders, revealed the presence of larger numbers of cytoplasmic particles corresponding to mitochondria than are found in the cells of more internal regions. Later concentrations of mitochondria in cells of the ciliated bands and gut, suggest a correlation between physiological activity and mitochondrial number. This correspondence, if it exists, however, need not bear a causal relationship to the differentiation of these regions.

The enumeration of mitochondria in intact cells of mesenchyme blastulae or young gastrulae, vitally stained with Nile Blue sulfate or Janus green, has not produced reliable results. Observations made either by focussing through the body wall or at one focal level, revealed approximately the same numbers of cytoplasmic inclusions absorbing the dyes in cells at all levels of the animal-vegetal axis. There are at least three reasons why the difficulties inherent in this method appear to render it incapable of yielding replicable results.

(1) The variability of mitochondria in size (0·5–3·0 or more micra) and shape (spherical, ovoid, rod-shaped, "dumb-bell"-shaped) is well known[1]. When they are stained with a dye (Nile Blue sulfate) which is also taken up by other cellular elements (yolk granules, pigment, fat droplets) in the same size range, it becomes very uncertain which of the dye-absorbing particles are mitochondria. Some observations of centrifuged eggs stained with Nile Blue sulfate, indicate only slight differences in the dye absorption of the different strata of granules. Janus green staining of intact eggs or embryos did not make mitochondrial identification much more reliable.

(2) Embryos stained with Nile Blue sulfate or Janus green have been carefully observed during flattening under the cover glass. Cells in the animal and vegetal regions of mesenchyme blastulae or gastrulae flatten at different rates, the latter having less resistance to pressure, and eventually rupturing sooner than the animal hemisphere cells under continued pressure. As the cells flatten, spreading of granules over a larger area occurs in the more vegetal regions, producing the image of fewer particles per unit area. Visible differences in dye uptake towards animal or vegetal poles in fixed embryos probably are also due to this unequal flattening of cells.

(3) Gradients of other substances in intact embryos, decreasing in a vegetal-animal direction, would tend to obscure mitochondria in more vegetal cells. Large numbers of yolk granules and oil droplets in vegetal cells "mask" other types of inclusions. When embryos are observed under pressure with phase microscope, cells which have been ruptured clearly show large numbers of dark bodies in the cytoplasm which can only be vaguely made out in less flattened cells. Similar observations have been made in vitally stained embryos.

In embryos fixed with Altmann's fluid and stained with fast-green and safranin, mitochondria are more readily identifiable than after vital staining. Observations of cells along the animal-vegetal axis of such embryos, with the highest power oil immersion lens, revealed no significant differences in numbers of mitochondria. As with the vitally stained specimens, however, mitochondrial counts were not replicable from embryo to embryo, for the reasons given above.

As development continues, there is a pronounced and rather rapid increase in mitochondria in the cells of differentiating gut structures (intestine, oesophagus, stomach and proctodeum). Continuing the tendency noted in the earlier ciliated cells, there is also an increase in mitochondria in cells forming the ciliated bands, extending across the body, and the arms of the developing pluteus. At the same time, there is a relative decrease in mitochondria in the cells of the body wall.

A quantitative method for counting mitochondria in homogenates[2] has been adapted for use with developmental stages of sea urchins[3]. Preliminary results with *L. pictus* indicate: (1) Mitochondria in an unfertilized egg occupy about 15% of the volume of the cell; (2) A rapid increase in mitochondria at the mesenchyme blastula stage followed by a decrease in mitochondria in gastrulae and young prism stages (about 25 h at 17°C). It is interesting that it is about this time when Gustaf-

[1] Zeiss 2 mm Apochromat.

[2] J. Harman, Stain Tech. *25*, 69 (1950).

[3] Tyler, Collecting Net *19*, 40 (1946).

[4] Fast green, FCF, Nat. Aniline & Chem. Co., New York. Cert. No. NCf9. 4% in 10% aniline.

[5] Safranine-O, Nat. Aniline & Chem. Co., New York. Cert. No. NS-7. 1 % in 50% ethyl alcohol.

[6] T. Gustafson and P. Lenicque, Exp. Cell Res. *3*, 251 (1952).

[7] Harvey, J. Exp. Zool. *102*, 253 (1946).

[8] Lansing and Hillier, cited in J. Hist. Cytochem. *1*, 265 (1954).

[1] *Cf.* J. Harman, Exp. Cell Res. *1*, 394 (1950).

[2] E. Shelton, W. C. Schneider, and M. J. Striebich, Exp. Cell Res. *4*, 32 (1953).

[3] Details of the method and data will be published elsewhere.

SON and LENICQUE[1] noted an overall decrease in mitochondria in *P. miliaris*.

J. R. SHAVER

Division of Biology, California Institute of Technology, Pasadena, March 15, 1955.

Zusammenfassung

Zytologische Untersuchungen an Embryonen von zwei Seeigelarten haben in bezug auf die Lokalisation der Mitochondrien keinen Unterschied zwischen animaler und vegetativer Hemisphäre ergeben. Vorläufige Versuche, mit Hilfe einer quantitativen Methode die Mitochondrien in Homogenaten zu zählen, ergaben eine starke Zunahme auf dem Stadium der Blastula und eine anschliessende Abnahme der Mitochondrienzahl pro Embryo.

[1] T. GUSTAFSON and P. LENICQUE, Exp. Cell Res. *3*, 251 (1952).

Factors Influencing the Fatty Acid Oxidation of Tumor Mitochondria with Special Reference to Changes in Spontaneous Mouse Hepatomas

WEINHOUSE and CHAIKOFF *et al.* gave a conclusive demonstration that slices of tumors are capable of oxidizing fatty acids[1]. Cell-free preparations of a number of tumors did not show this property, however[2]. It is the object of this short communication to report on some of the factors influencing the oxidation of fatty acids by tumor mitochondria *in vitro*, and especially on changes in this respect occuring in mitochondria from spontaneous hepatomas. So far as we are aware, the present data furnish the first positive evidence of fatty acid oxidation by tumor mitochondria.

The hepatomas studied arose in a high incidence in two year old female F_1(♀ C_{57} Black × ♂ C_3He) mice[3] which were kindly put at our disposal by Dr. O. MÜHLBOCK.

The tumors were always divided in two groups, consisting of hepatomas of less than 1 g, which we shall call early stage tumors, and tumors weighing 2–10 g, which are designated here as fully developed tumors. Mitochondria, prepared from both specimens in isotonic (0·25 m) sucrose[4], were washed twice in a volume of cold isotonic sucrose equal to twice the original tissue weight, and the suspension prior to incubation–the preliminary suspension– was made in a (0·13 m) KCl-(0·013 m) phosphate buffer (pH 7·4). After the mitochondrial suspension was pipetted into Warburg flasks containing the proper additions (Table I), only the mitochondria from the early stage tumors showed an oxidation of octanoate with α-oxy caproate and L-malate as "sparkers" of fatty acid oxidation[5].

In a previous publication[6], it was shown that mitochondria prepared from many mouse tumors exhibit a marked "adenosine triphosphatase (ATPase) activity", as measured by the complete inhibition of fatty acid oxidation of normal liver mitochondria after addition of these tumor mitochondria. Most tumor mitochondria showed this high "ATPase activity" irrespective of the fact whether the preliminary suspension was made in isotonic sucrose or in KCl-phosphate buffer. At the same time it was found that no oxidation of octanoate took place by these mitochondria *per se* after addition of NaF (0·01 m) diphosphopyridine nucleotide (10^{-4} m) and coenzyme A (10 γ).

The mitochondria from the fully developed hepatomas also showed a high "ATPase activity", when the preliminary suspension was made in the KCl-phosphate buffer, but, when isotonic sucrose devres as the suspending medium, only a moderate or slight "ATPase activity" was recorded. This was more or less anticipated in view of the apparent lack of octanoate oxidation by the mitochondria from the preliminary KCl-phosphate suspension. After addition of fluoride–a well-known inhibitor of ATPase–a small oxygen consumption resulting from octanoate oxidation could sometimes be seen. Mitochondria from the preliminary sucrose suspension were somewhat variable in their oxidative behaviour, sometimes showing an oxygen consumption with octanoate as the substrate. Isolation of the mitochondria with isotonic sucrose containing 0·001 ethylenediamine tetraacetate (versene) resulted in an enhanced oxidation.

The "ATPase activity" of the mitochondria from the early stage tumors was considered as small or absent, because even the mitochondria from the preliminary KCl-phosphate suspension were capable of oxidizing octanoate; this was confirmed by measurements of the "ATPase activity" in the way indicated.

The absence of "ATPase activity" does not necessarily mean that tumor mitochondria in general are able to effect fatty acid oxidation. Mitochondria prepared from two different transplanted interstitial cell tumors of the mouse testis never showed such ATP splitting activities as would influence the fatty acid oxidation of normal liver mitochondria appreciably. The mitochondria from one of these tumors, prepared either with or without versene, were, however, unable to oxidize fatty acids under the conditions of the present experiments. The mitochondria from the other testis tumors, prepared with sucrose-versene, showed a vivid oxidation of octanoate. Another example may be mentioned of a transplanted granulosa cell tumor of the mouse ovary. Mitochondria prepared from this tumor with isotonic sucrose and suspended in KCl-phosphate buffer had a moderate or marked "ATPase activity". When the preliminary suspension was made in isotonic sucrose and added to normal liver mitochondria, no influence on the fatty acid oxidation of the liver mitochondria could be detected, but the tumor mitochondria *per se* showed no octanoate oxidation. When the whole procedure of preparing the tumor mitochondria was carried out with 0·001 m versene included in the isotonic sucrose solution, a very large oxygen consumption resulted from octanoate oxidation.

Changes in the mitochondrial nitrogen content of the spontaneous hepatomas were also noted. The nitrogen content of the mitochondria per 100 mg fresh tissue of the early stage hepatomas was always found to be higher than the corresponding values of the fully developed tumors, e.g. 0·39 against 0·28 mg N on the average. This may be the result of the greater blood content of the latter. The division of the spontaneous hepatomas into the two groups mentioned was merely

[1] S. WEINHOUSE, R. H. MILLINGTON, and C. E. WENNER, Cancer Res. *11*, 845 (1951). – D. D. CHAPMAN, G. W. BROWN Jr., I. L. CHAIKOFF, W. O. DAUBEN, and N. O. FAUSCH, Cancer Res. *14*, 372 (1954).

[2] C. G. BAKER and A. MEISTER, J. Natl. Cancer Inst. *10*, 1191 (1950).

[3] O. MÜHLBOCK, unpublished.

[4] W. C. SCHNEIDER and G. H. HOGEBOOM, J. Biol. Chem. *183*, 123 (1950).

[5] N. WATERMAN, C. J. BOS, and T. J. BARENDREGT, Enzymologia *15*, 307 (1952). – P. EMMELOT and C. J. BOS, Enzymologia *17*, 13 (1954).

[6] P. EMMELOT and C. J. BOS, Biochim. Biophys. Acta *16*, 620 (1955).

Table I.–Oxidation of Octanoate by Tumor Mitochondria and by Liver Mitochondria in the presence of Tumor Mitochondria. Additions: octanoate ($1{\cdot}25 \times 10^{-3}$ m), α-oxy caproate (3×10^{-3} m), L-malate (7×10^{-5} m), ATP (7×10^{-4} m), cytochrome c (10^{-5} m), diphosphopyridine nucleotide (DPN, 10^{-3} m), Mg^{++} (5×10^{-3} m), in a total volume of 1·6 ml (0·06 m) KCl-(0·013 m) phosphate buffer (pH 7·4) including 0·6 ml suspension of tumor mitochondria in KCl-phosphate buffer (KCl-P), or 0·25 m sucrose ± versene (0·001 m). NaF was added in one experiment in a final concentration of 0·01 m. In the "ATPase assay" (lower part of table) 0·3 ml suspension of liver mitochondria in KCl-phosphate buffer and 0·3 ml suspension of tumor mitochondria in KCl-phosphate or 0·25 m sucrose ± versene were added, DPN and malate were omitted. Oxygen consumption was calculated as the difference between oxygen uptake in the presence of octanoate + α-oxy caproate (+ DPN) and that of α-oxy caproate (+DPN). Protein-N was determined by a micro-Kjehldahl procedure. In the experiments in which tumor and liver mitochondria were incubated together, the N-content of the former is listed in the table. The N-content of the latter was always the same as that of the liver mitochondria incubated separately.

Mitochondria from	preliminary suspension	mg N per flask	$\mu l O_2$ consumed per flask after 60 min
hepatomas:			
"early stage" (I)			
Ia	KCl-P	2·18	177
Ib	KCl-P	2·37	140
"fully developed"(II)			
IIa	KCl-P	2·28	−3
IIb	KCl-P	1·81	−4
IIb+F^-	KCl-P	1·81	23
granulosa cell tumor (III)			
IIIa	sucrose	1·06	0
IIIa	sucrose+versene	0·76	246
mouse liver (IV)			
IVa		0·80	60
IVa+Ic	KCl-P	1·68	100
IVb		1·38	117
IVb+Id	sucrose	0·78	140
IVb+IIc	KCl-P	0·89	10
IVb+IIc	sucrose	0·73	60
IVc		1·42	121
IVc+IId	sucrose	1·73	106
IVd		1·26	124
IVd+IIIb . . .	KCl-P	1·01	59
IVe		1·26	115
IVe+IIIc . . .	KCl-P	0·78	14
IVf		0·98	117
IVf+IIIa . . .	sucrose	1·06	130
IVf+IIIa . . .	sucrose+versene	0·76	190

made on account of the weight of the tumors. The results reported were confirmed in more than 25 experiments, only a very few exceptions being noted. It can be concluded that the mitochondria from the fully developed hepatomas are more liable to damage *in vitro*, by which latent ATPase activities become activated. By splitting ATP the forming of the acyl-coenzyme A bond, a necessary requisite for fatty acid oxidation, is precluded.

P. Emmelot and C. J. Bos

Department of Biochemistry, Antoni van Leeuwenhoekhuis, the Netherlands Cancer Institute, Amsterdam, April 14, 1955.

Zusammenfassung

Mitochondrien aus kleinen und grossen Spontanhepatomen bei $F_1(C_{57}Bl \times C_3He)$ Mäusen weisen verschiedene Adenosintriphosphataseaktivitäten auf, was sich 1. an der Hemmung der Fettsäureoxydation normaler Lebermitochondrien nach Zugabe von Tumormitochondrien und 2. an der Fettsäureoxydation der Hepatommitochondrien selbst zeigen lässt. Die Mitochondrien gewisser Tumoren vermochten Caprylsäure nur zu oxydieren, wenn die Adenosintriphosphatase möglichst wenig aktiviert war durch Zugabe von Verseen zum Aufarbeitungsmedium.

Sur la présence de β-alanine dans les tissus des plantes supérieures

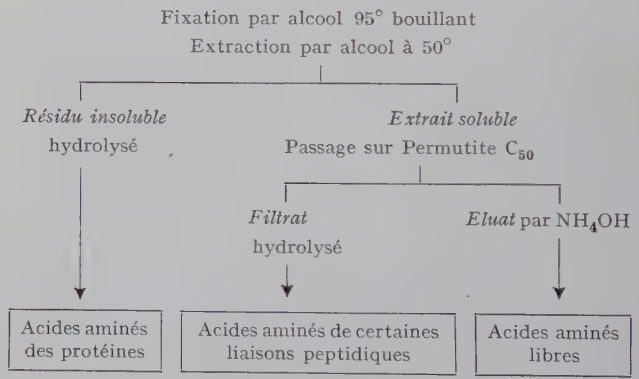
La β-alanine a été identifiée parmi les acides aminés libres des végétaux supérieurs par plusieurs auteurs: Steward et Thompson[1] dans des tissus variés; Hunt[2] dans les racines et les nodules de légumineuses, Auclair et Maltais[3] dans les extraits de pois.

Par contre, au cours de travaux portant sur les protéines des tissus végétaux, il n'a jamais été décelé de β-alanine dans les hydrolysats. Peu de recherches ont porté sur les acides aminés constituant des polypeptides.

Cependant, Virtanen et Miettinen[4] ont identifié la β-alanine chez le Pois (feuilles), à la fois parmi les acides aminés libres, et comme constituant des polypeptides.

Au cours de travaux d'orientations différentes sur le métabolisme protidique, nous avons rencontré la β-alanine dans certains extraits obtenus à partir de feuilles de blé et de *Bryophyllum daigremontianum* Berger (M.L.C.) et de divers tissus végétaux cultivés *in vitro*: tissus normaux de racines de scorsonère, tissus de Crown-gall de scorsonère, tissus de Crown-gall de topinambour (C.L.).

Les techniques de séparation employées sont résumées dans le schéma suivant:



Le passage sur la Permutite C_{50} (méthode de Boulanger et Biserte[5]) permet de retenir les acides aminés

[1] F. C. Steward et J. F. Thompson, Ann. Rev. Plant. Physiol. *1*, 233 (1950).
[2] G. E. Hunt, Amer. J. Bot. *38*, 452 (1951).
[3] J. L. Auclair et J. B. Maltais, Nature *170*, 1114 (1952).
[4] A. I. Virtanen et J. K. Miettinen, Biochem. Biophys. Acta *12*, 181 (1953).
[5] P. Boulanger et G. Biserte, Bull. Soc. Chim. Biol. *33*, 1930 (1951).

libres et certains polypeptides courts. D'autres substances (polypeptides longs, glucides, etc.) traversent la colonne et constituent le filtrat, qui, après hydrolyse, fournit les acides aminés de corps solubles contenant des liaisons peptidiques. L'élution des acides aminés libres retenus par la résine est faite par NH_4OH N. Les hydrolyses sont faites par HCl 6 N à 100° pendant 24 h. Des distillations répétées sous vide permettent d'éliminer la plus grande partie de HCl. La purification s'effectue ensuite par passage sur permutite C_{50} et élution comme précédemment. La séparation est faite par chromatographie sur papier à 2 dimensions selon le système de DENT[1]. La β-alanine apparaît en bleu clair au réactif à la ninhydrine.

Les résultats relatifs à la présence de β-alanine dans les tissus étudiés sont indiqués dans le tableau suivant:

	Acides aminés libres	*Fraction peptidique*	*Fraction protéique*
Racines de scorsonère . . .	+	+	–
Crown-gall scorsonère . . .	–	+	–
Crown-gall topinambour .	+	+	–
Feuilles de blé	–	+	–
Feuilles de bryophyllum. .	–	+	–

On retrouve ainsi les données antérieurement acquises: présence occasionnelle de β-alanine parmi les acides aminés libres, absence totale parmi les acides aminés d'origine protéique. De plus, on peut remarquer qu'elle est toujours présente dans la fraction peptidique. D'après VIRTANEN[2] la β-alanine libre proviendrait de la décarboxylation de l'acide aspartique. VIRTANEN et MIETTINEN[3] pensent aussi que, dans la fraction peptidique, la β-alanine serait liée à des sucres réducteurs par son groupe NH_2 devenu plus réactif du fait de son éloignement du groupe carboxyle. On peut émettre également l'hypothèse qu'il s'agit de la β-alanine constitutive de l'acide panthoténique entrant lui-même dans la composition du «coenzyme A».

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Laboratoire de Photosynthèse, Centre national de la Recherche scientifique, Gif-sur-Yvette, le 28 mai 1955.

Summary

β-alanine has always been found in peptide hydrolysates of various higher plants, but never in protein hydrolysates. An assumption that the β-alanine so found is a constituent of coenzyme A has been suggested.

[1] C. E. DENT, Biochem. J. *43*, 169 (1948).
[2] A. I. VIRTANEN, Nature *142*, 674 (1938).
[3] A. I. VIRTANEN et J. K. MIETTINEN, Biochem. Biophys. Acta *12*, 181 (1953).

Histochemical Demonstration of Reductase Activity in the Nucleus of Chicken Erythrocyte[1]

Recently RUBINSTEIN and DENSTEDT[2] have demonstrated the presence of Krebs cycle enzymes in the nuclei of chicken erythrocytes. The work of the above investigators was criticized by STERN and TIMONEN[1] who were concerned with a possible contamination of the nuclear preparation by postulated cytoplasmic particles or with a possible absorption by the isolated nuclei of cytoplasmic enzymes made soluble through freezing-thawing techniques. The interesting original work and the subsequent criticism led us to examine these nuclei histochemically for the presence of enzymes concerned with reductase activity.

Histochemical work, using basic techniques previously described[2], confirms the actual presence of general reductase and specifically of succinic dehydrogenase activity in the nuclei of chicken erythrocytes.

Four tetrazolium salts were tested: 2, 3, 5-triphenyl tetrazolium chloride (TTC); 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INTC); 2, 2′-(p-diphenylene)-bis-2-(3, 5-diphenyl) tetrazolium chloride, or neotetrazolium (NT); and 3, 3′-dianisole-bis-4, 4′-(3, 5-diphenyl) tetrazolium chloride, or blue tetrazolium (BT)[3].

The first two are monotetrazolium, the latter ditetrazolium compounds. The concentrations used were 1 mg/ml. The different salts varied not as much in the final results as in the time of incubation required to obtain formazan pigment. The INTC was preferred as it gave faster reduction and more minute granulation. Purification of the tetrazolium salts by removal of contaminating metal by previous treatment with a chelating agent, diphenylthiocarbazone, was very effective in decreasing the time of incubation, particularly in the case of the INTC.

Different methods, whose characteristics will be discussed elsewhere, were used in order to achieve satisfactory histochemical results. The reaction was performed both in intact cells as well as in nuclei, which were isolated by freezing and thawing. Freezing and thawing were used for demonstration of succinic dehydrogenase as freezing destroys the so called "endogenous" dehydrogenase.

Potassium cyanide and versene (tetrasodium salt of ethylenediaminotetraacetic acid) at concentrations from 0·01 *M* to 0·1 *M* proved to be very effective on the final histochemical result, probably by affecting the membrane permeability[4] and enhancing the enzymatic activity[5].

The specificity of the mechanism involved in the reduction was confirmed by absence of formazan formation in the case of succinic dehydrogenase on leaving out the succinate or on adding fluoride or malonate in addition to succinate.

Results under each test condition were highly reproducible and the number of formazan crystals per cell was fairly uniform. The crystals were localized at the inner surface of the nuclear membrane; and only very rarely were crystals encountered at the center of the nucleus. No tetrazolium reduction was observed with or without succinate in the cytoplasm of the intact cell, as well as in the supernatant fluid from which nuclei had been isolated.

[1] This work was supported in part by a Research Grant from the National Cancer Institute, U. S. Department of Health, Education and Welfare, and in part by an Institutional Grant to the Detroit Institute of Cancer Research from the American Cancer Society.

[2] D. RUBINSTEIN and O. F. DENSTEDT, J. Biol. Chem. *204*, 623 (1953).

[1] H. STERN and S. TIMONEN, J. Gen. Physiol. *38*, 41 (1954).

[3] B. PEARSON and V. DEFENDI, J. Histochem. Cytochem. *2*, 248 (1954). – V. DEFENDI and B. PEARSON, J. Histochem. Cytochem. *3*, 61 (1955).

[3] Obtained from the Pal Chemicals Limited, London, England.

[4] S. R. MERKEL and W. J. NICKERSON, Proc. Nat. Acad. Sci. *39*, 1008 (1953).

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This is the first time, as far as it is known to the authors, that succinic dehydrogenase has been demonstrated histochemically under conditions in which no mitochondrial bodies are present. It is most remarkable that, in a cell whose cytoplasm has assumed a unique, highly specialized function, the nucleus, which is in a continuous resting phase, displays metabolic activities that are usually associated with the cytoplasm in other tissues.

V. Defendi and B. Pearson

Detroit Institute of Cancer Research, Detroit 1, Michigan, April 26, 1955.

Zusammenfassung

Dehydrase- und Succinodehydraseaktivität werden mittels histochemischer Methode durch Tetrazoliumsalze in Kernen der Hühnererythrocyten dargestellt. Das Vorhandensein der Formazankristalle beschränkt sich vor allem auf die innere Oberfläche der Kernmembrane. Die Bedeutung dieser Entdeckungen im Zusammenhang mit dem Succinodehydrase-Mitochondrien-Verhältnis wird kurz besprochen.

Etude histochimique de l'action des rayons X sur les cholinestérases

On sait depuis longtemps[1] que les globules rouges possèdent de l'acétylcholinestérase (AChE = cholinestérase spécifique ou vraie). Mais ce n'est que depuis les travaux de Zajíček et de Rogister[2] qu'on connaît la présence de cet enzyme dans les éléments qui sont à l'origine des cellules sanguines, à savoir les érythroblastes et les mégacaryocytes, et aussi dans les thrombocytes[3].

Chez l'homme, on trouve cette estérase presque exclusivement dans les érythrocytes, où elle serait localisée sur la membrane[4], et dans les érythroblastes, tandis que chez le chat, elle se trouve dans le protoplasme des mégacaryocytes et des plaquettes. Chez la souris, animal qui a servi à nos expériences, l'enzyme est peu actif dans la lignée érythrocytaire et fort actif dans la lignée thrombocytaire.

On ignore le rôle de l'AChE dans les éléments sanguins. On suppose qu'elle intervient pour maintenir la perméabilité de la membrane cellulaire, car l'inhibition par la physostigmine provoque une modification de la perméabilité et de l'échange des ions sodium et potassium[5].

En partant du fait que la perturbation de perméabilité cellulaire est une des premières manifestations du syndrome d'irradiation[1], nous avons cherché à déterminer l'influence des rayons X sur les cholinestérases des éléments hématopoïétiques et des cellules du sang. Dans ce dernier, l'AChE des hématies échappe à l'analyse histochimique, sans doute à cause de l'état dispersé de l'enzyme dans ces éléments. D'autre part, les plaquettes sont moins favorables à l'étude histochimique que les mégacaryocytes. Nous avons donc examiné l'activité acétylcholinestérasique des mégacaryocytes dans la rate et la moelle osseuse chez des souris de race C3H (mâles de 6 mois). Dans une série d'expériences, nous avons observé aussi l'activité de cet enzyme dans les plaques motrices du muscle strié. Quant à la cholinestérase (ChE = cholinestérase non spécifique ou pseudo-cholinestérase), celle du sérum ne peut être mise en évidence par notre technique, et nous avons dû utiliser des coupes de divers organes (foie, pancréas, prostate) pour définir l'effet de l'irradiation sur cet enzyme.

Les animaux ont été irradiés au moyen d'un appareil «Siemens» (250 kV, 20 mA, 0,5 Cu, champ 15 × 15 cm, intensité 80 r/min) avec contrôle au dosimètre «Siemens». Les doses d'irradiation et les temps de survie accordés aux animaux sont indiqués dans le graphique. Certaines souris ayant reçu 800 ou 600 r ont été laissées en vie plus longtemps: jusqu'à 20 jours après l'irradiation. La dose mortelle pour nos souris est de 750 r.

La détermination d'activité cholinestérasique est faite suivant la méthode de Koelle modifiée par Gerebtzoff[2]. Pour chaque organe, les coupes ont été incubées en présence d'acétylthiocholine ou de butyrylthiocholine durant 30 à 120 min. L'estimation d'activité enzymatique se fait d'après l'intensité de la coloration. Elle est, par conséquent, subjective, et ne donne qu'une idée imprécise des variations quantitatives.

L'ensemble des résultats est indiqué dans le graphique.

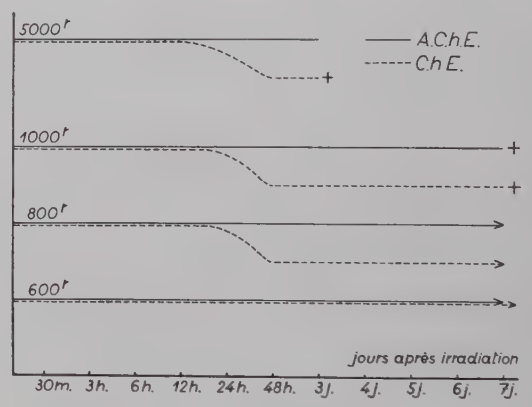

Fig. 1.

L'AChE des mégacaryocytes de la rate et de la moelle osseuse et aussi celle des plaques motrices est très résistante aux rayons X, même à la dose de 5000 r. On n'a pas constaté de diminution de leur activité même quand les mégacaryocytes présentent de grandes altérations dans leur structure cellulaire (vacuolisation et désintégration du noyau). L'activité de l'AChE disparaît en même temps que s'éliminent les débris de mégacaryocytes, c'est-à-dire au bout de 2 jours pour 5000 r, vers le 5e jour pour 1000 r, et au-delà d'une semaine pour 800 r. Après une dose de 600 r, les mégacaryocytes et l'activité enzymatique persistent. Quelle que soit la dose,

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[2] J. Zajíček et N. Datta, Acta Haemat. *9*, 115 (1953). – J. Zajíček, B. Sylven et N. Datta, J. Histochem. and Cytochem. *2*, 115 (1954). – J. Zajíček, Acta Haemat. *12*, 238 (1954). – G. Rogister, C. r. Soc. Biol. *148*, 1900, 1910 (1954).

[3] J. Zajíček et N. Datta, Acta Haemat. *9*, 115 (1953). – J. Zajíček, B. Sylven et N. Datta, J. Histochem. and Cytochem. *2*, 115 (1954). – J. Zajíček, Acta Haemat. *12*, 238 (1954). – G. Rogister, C. r. Soc. Biol. *148*, 1900, 1910 (1954). – K. B. Augustinsson, N. Datta, M. Grahn et J. Zajíček, Nature *169*, 800 (1952). – J. Zajíček et N. Datta, Acta haemat. *7*, 39 (1952).

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[5] K. B. Augustinsson in *The enzymes* by J. B. Sumner and Myrbäck, vol. 1 (Academic Press, New-York 1950). – W. C. Holland et M. E. Greig, Amer. J. Physiol. *162*, 610 (1950). – M. E. Greig et W. C. Holland, Arch. Biochem. *23*, 370 (1949).

[1] Z. M. Bacq et P. Alexander, *Principes de Radiobiologie* (Masson, Paris 1955).

[2] M. A. Gerebtzoff, Acta Anat. *19*, 366 (1953).

l'intensité de la réaction histochimique ne diminue pas dans les plaques motrices.

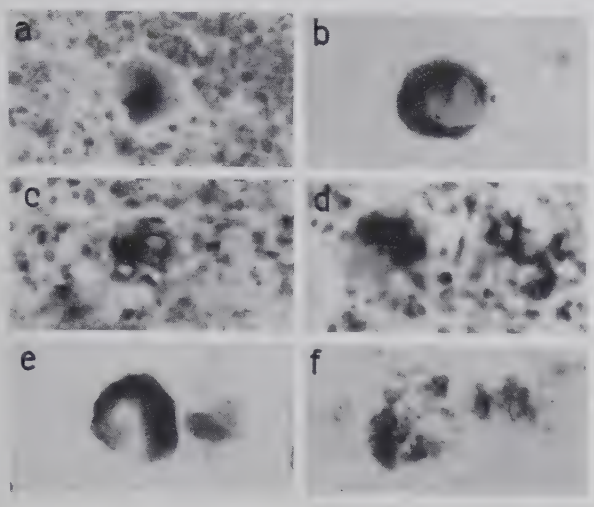

Fig. 2

Mégacaryocytes dans la rate de la souris. *a* normal; coloration à l'hémalun-éosine. *b* normal; AChE. *c* mégacaryocyte vacuolisé 24 h après 5000 r; coloration à l'hémalun-éosine. *d* un mégacaryocyte peu altéré et un autre en désintégration 24 h après 5000 r; même coloration. *e* un mégacaryocyte peu altéré et un autre en voie de disparition 24 h après 5000 r; AChE. *f* deux mégacaryocytes en désintégration 24 h après 5000 r; AChE. Grossissement: 450 ×. Incubation en présence d'acétylthiocholine: 90 à 120 min à pH 6,2.

On ne constate pas de diminution de l'activité de la ChE du foie immédiatement après l'irradiation. Cette activité commence à baisser plus tard: vers 12 h après 5000 r; cette chute est plus accentuée vers 24 h et 48 h après les doses de 5000 r, 1000 r et 800 r, comme le montre le graphique. On n'a constaté aucune diminution même 15 ou 20 jours après une dose de 600 r. La ChE du pancréas et de la prostate réagissent de la même façon.

Nos résultats s'accordent avec ceux de CONARD[1] sur la ChE de l'intenstin, obtenus en utilisant la technique colorimétrique d'après HESTRIN, et avec ceux de BURN et collaborateurs[2] sur l'AChE et la ChE du foie et de l'intestin, au Warburg. Il est difficile de faire une comparaison avec les travaux de BAGLIONI[3] et de KWIATKOWSKI[4] à cause des différences de conditions expérimentales.

En conclusion, nous pouvons dire que l'AChE des mégacaryocytes de la rate et de la moelle osseuse et aussi celle des plaques motrices est très résistante aux rayons X. On obtient les mêmes résultats pour la ChE du foie, du pancréas et de la prostate, dont l'activité n'est pas diminuée immédiatement après l'irradiation, mais baisse légèrement après 12 h et surtout 48 h pour les doses de 5000 r, 1000 r et 800 r. Il est probable que cela est dû à la diminution des synthèses enzymatiques dans le foie, organe qui souffre profondément à la suite de l'irradiation[5].

La grande résistance des cholinestérases et surtout de l'AChE au rayonnement X semble indiquer que les modifications de perméabilité des membranes cellulaires qui conditionnent les radiolésions ne sont pas liées à des modifications de ces enzymes.

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Summary

Using a histochemical method, we have observed the cholinesterase activity in mice after X irradiation. Both types of cholinesterases were examined: AChE (specific ChE) of megacaryocytes of spleen and bone marrow, and AChE of motor endplates; and non-specific ChE of liver, pancreas and prostate. There is no inactivation of AChE even after 5000 r. ChE is also very resistant: activity is slowed down only 12 h or even a few days following irradiation and the inactivation remains slight. We suppose that the great alterations in cellular permeability caused by irradiation are not correlated with these enzymes.

Über eine bakterielle Vorverdauung und eine proteolytische Hauptverdauung im Darm der Nashornkäferlarve (*Oryctes nas.* L.)

WIEDEMANN[1] hat nachgewiesen, dass sich im Enddarm (Saccusabschnitt) der Orycteslarve Zellulose abbauende Bakterien befinden. SCHLOTTKE[2] hat bei anderen im Holz fressenden Käferlarven ein proteolytisches Ferment im Mitteldarm mit einem Wirkungsoptimum bei pH etwa 9,3 gefunden.

Zunächst konnten wir die Feststellungen beider Autoren bei der Larve des Nashornkäfers bestätigen. Die Annahme WIEDEMANNS, ein proteolytisches Ferment werde im sauren Milieu des Saccus aktiviert und übe dort eine verdauende Wirkung auf die Zellulose abbauenden Bakterien aus, hat bereits SCHLOTTKE im Zusammenhang mit seinem Mitteldarmferment in Frage gestellt.

Obwohl GOETSCH[3] bei einigen Darmbakterien beherbergenden Insekten durch Einbringen von Darmteilen in Bakterienkulturen eine auflösende Wirkung erzielte, blieb im hier vorliegenden Falle die Frage, ob überhaupt eine Eiweissverdauung über die bakterielle Tätigkeit vorhanden ist, offen. Ferner blieb unklar, wie und wo im positiven Fall die Bakterien unter den Einfluss des proteolytischen Fermentes gelangen.

Einfluss von Mitteldarmextrakt auf Bakterien. Der Extrakt wurde als Glyzerin-Wasser-Auszug, EK-gefiltert durch Schott G3/G5, auf Kulturen der Darmbakterien in Omelianskylösung zur Einwirkung gebracht. Nach dreitägiger Einwirkung bei 35°C und pH über 7 waren sämtliche Bakterien aufgelöst, während die Kontrollen unverändert blieben. Bei Überimpfung von behandelten und unbehandelten Kulturen auf Agarplatten wurde Koloniewachstum erzielt. Das Ferment hatte offenbar die Sporen in ihrer Keimfähigkeit nicht beeinträchtigt.

N-Quelle. Wir stellten fest, dass der N-Gehalt des Futters (Sägemehl) 0,11% beträgt, der N-Gehalt des Nahrungsbreies im Saccus aber das 10fache davon ausmacht. Der N-Gehalt des Kotes liegt mit 0,315% noch wesentlich über dem N-Gehalt des Futters. Auf einen

[1] R. A. CONARD, Amer. J. Physiol. *170*, 418 (1952).
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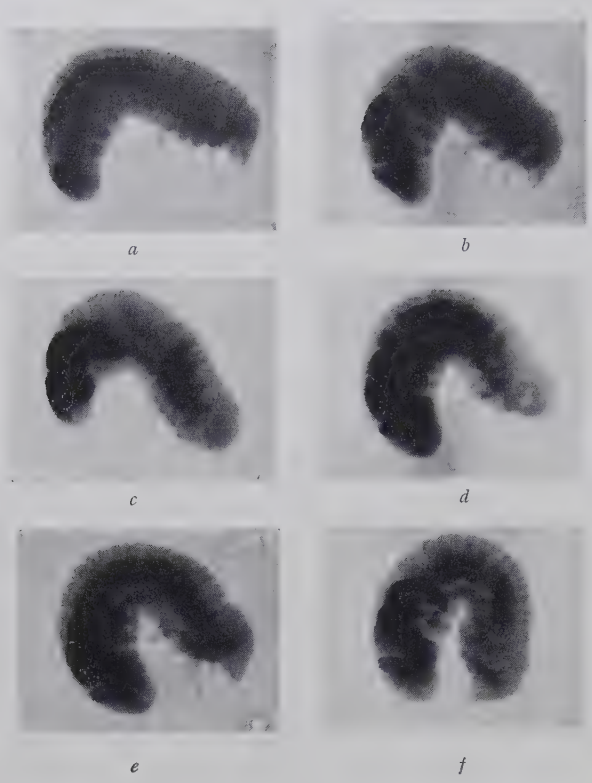
[2] E. SCHLOTTKE, Zool. Jb. *61*, 88 (1945).
[3] W. GOETSCH, Österr. Zool. Z. *1*, 58 (1946).



6 Stadien aus einem Röntgenfilm über die Nahrungspassage im Darmkanal der Nashornkäferlarve. *a* 29. April, 17.45 Uhr; *b* 30. April 11.15 Uhr; *c* 2. Mai, 9.50 Uhr; *d* 4. Mai, 21.10 Uhr; *e* 6. Mai, 23.00 Uhr; *f* 8. Mai, 17.40 Uhr. Bei *c* ist der gesamte kontrastmittelhaltige Nahrungsbrei im Saccus; bei *d* ist der Rücktransport im Mitteldarm deutlich zu sehen.

primären Stickstoffgewinn erfolgt somit ein erneuter Verbrauch. Das auffallende Faktum ist, dass die Larve des Nashornkäfers im Kot die dreifach grössere N-Menge ausscheidet, als sie mit dem Futter aufgenommen hat. Parallel hierzu ergaben die kalorimetrischen Verbrennungen für das Futter einen Energiegehalt von 4,09 Cal/1 g Futter (Streuung 4,2–4,03 Cal/1 g) und für den Kot 4,30 Cal/1 g Kot (Streuung 4,42–4,23 Cal/1 g). Keimzählungen im Mitteldarm, im Saccus und im Kot ergaben eine auffallende Verschiedenheit in den Populationszahlen. Für den Mitteldarm fanden wir 188 Millionen Keime pro 1 cm³, für den Saccus 470 Millionen Keime und für den Kot 76 Millionen Keime pro 1 cm³. Wie bei den N-Gehaltsbestimmungen zeigen auch diese Zahlen, dass die N-Quelle in der Bakterienvermehrung im Saccus zu sehen ist. Ob die Bakterien des Saccus hauptsächlich die im Holz vorliegende Zellulose oder andere Zucker (Pentosane) zum Aufbau ihres Körpereiweisses verwenden, konnten wir noch nicht klären. Die Untersuchungen darüber werden fortgesetzt.

Wirkungsort des Fermentes. Die vorstehenden Befunde liessen uns an den Verdauungsmodus bei Wiederkäuern denken und an die Möglichkeit eines Rücktransportes von Nahrungsbrei und Bakterienflora im Verdauungstraktus der Orycteslarve zum Mitteldarm.

Röntgenologisch haben wir den Weg, den die Nahrung im Darm zurücklegt[1], verfolgt (Abb.). Die Aufnahmen zeigen, dass der Darmbrei (Sägemehl mit Bariumsulfat als Kontrastmittel) zunächst in etwa 17 h durch den Mitteldarm hindurch in den Saccus gelangt und dort ungefähr 3–4 Tage verweilt. Hierauf erfolgt ein fraktionierter Rücktransport in den Mitteldarm. Noch nach 7 Tagen konnte bariumsulfathaltiger Nahrungsbrei im Mitteldarm festgestellt werden[1].

Damit ist nachgewiesen, dass in der Tat die Orycteslarve das Wiederkäuen von Zellulose im Insektenreich verwirklicht. Die bakterielle Vorverdauung ist im Saccus lokalisiert, während die proteolytische Hauptverdauung offenbar mit dem Wiederkauakt verknüpft ist und nur im Mitteldarm stattfindet.

Marlies Roessler

Zoologisches Institut der Johannes-Gutenberg-Universität Mainz, den 15. Juni 1955.

Summary

The larva of *Oryctes nas.* L. shows a bacterial predigestion in the rectal-saccus. The main breakdown of proteins takes place in the middle gut, closely connected with the act of regurgitation.

[1] E. T. Nielsen, Ent. Medd. *23*, 255 (1943).

[1] Th. Wildbolz, Mitt. Schweiz. Entomolog. Ges. *27*, 193 (1954). Th. Wildbolz hat bei der Larve von *Melolontha melolontha* L. die Verweildauer der Nahrung im Darmkanal untersucht. Er findet eine sehr variable Passagegeschwindigkeit und im Mitteldarm Gasblasen, die er als beim Schlingakt aufgenommene Luft interpretiert. Auf Grund der eigenen Befunde ist anzunehmen, dass es sich dabei um Gärgase aus dem Saccus handelt.

Fat Metabolism in Various Forms of Experimental Obesity. VI. Instantaneous Lipogenesis in Hypothalamic Obese Mice[1]

In a series of reports[2] it was shown that various forms of experimental obesity differed as far as lipogenesis was concerned. Mice with the hereditary obese-hyperglycemic syndrome incorporated more acetate into fatty acids than did their non-obese siblings not only, as predictable, when both groups were fed *ad libitum*, but also when the obese were pair-fed with the controls, when both groups were underfed in similar proportion and even when both groups were fasted. By contrast, goldthioglucose obese mice and hypothalamic hyperphagic rats only incorporated more acetate into fat than their controls in the measure that they were allowed to overeat. The first type of obesity (increased lipogenesis under fasting, etc., conditions) has been termed "metabolic" obesity, the second type (increased lipogenesis dependent upon increased caloric intake) has been termed "regulatory obesity"[3]. Since lipogenesis in genetic obesity and goldthioglucose obesity had been studied in mice, and hypothalamic obesity in rats, species differences could vitiate the comparison between the three forms. Hypo-

[1] Supported in part by grants-in-aid from the National Institute of Arthritis and Metabolism (Grant No. A49C2R), National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare; Sugar Research Foundation, New York City; Kellogg Co., Battle Creek, Michigan; and the J. M. Kaplan Fund, Inc., New York.

[2] M. W. Bates, J. Mayer, and S. F. Nauss, Amer. J. Physiol. *180*, 304 (1955). – M. W. Bates, C. Zomzely, and J. Mayer, Amer. J. Physiol. *181* 187 (1955). – J. Mayer, N. C. Hagman, N. B. Marshall, and A. J. Stoops, Amer. J. Physiol.(in press, June 1955).

[3] J. Mayer, Proc. IIIrd Internat. Nutrition Congr., Voeding, *16*, 62 (1955).

C^{14}-acetate Incorporation into Carcass and Liver Fatty Acids in Hypothalamic Obese Mice and Controls

Nutritional Status	Type *	Body Weight g	Liver Weight g	C^{14}-Incorporation		
				Total	Carcass	Liver
Nonfasted	Obese	54·7 ± 5·7	2·228 ± 0·78	1266 ± 236	930 ± 32	384 ± 43
Nonfasted	Nonobese	34·0 ± 1·3	1·875 ± 0·06	3705 ± 561	318 ± 17	52 ± 17
Fasted	Obese	59·8 ± 6·6	2·965 ± 0·40	231 ± 69	205 ± 28	42 ± 13
Fasted	Nonobese	28·1 ± 2·0	1·810 ± 0·11	249 ± 23	187 ± 42	27 ± 6

* 8 animals of each type. Incorporation is expressed as percentage counts retained × 10^3.

thalamic obesity has since been achieved in mice[1]. It was therefore logical to repeat the most informative lipogenesis experiment using hypothalamic hyperphagic mice rather than rats, to check that the characteristics previously described were typical of the syndrome itself and not limited to one species.

Sixteen 4 month old female mice were used. The experimental animals were 8 hypothalamic obese swiss mice, weighing from 50 to 65 g and their unoperated controls, weighing 27 to 36 g. The obese animals had been operated according to the procedure described previously[1]. The obese mice used in the "non-fasted" experiment had been operated 4 months previously and were putting weight on slowly. The obese mice used in the "fasted" (16 h fast) experiment had been operated 1 month previously and were still putting on weight rapidly. (Thus, the demonstrated failure to demonstrate an increased acetate incorporation in the fasted state, contrasting with the picture in the fed state could not be attributed to the fasted animals being in a less "dynamic" form of obesity[2].) All mice were maintained in individual cages, in an air-conditioned room and were fed Purina Laboratory Chow *ad libitum*. The animals were injected with 0·5 cm^3 of saline solution containing 0·075 mg of C^{14}-carboxyl-labeled acetate containing 1 500 000 cpm in the case of the obese animals, 150 000 cpm in the case of the nonobese. They were killed half an hour later and the procedure previously described[3] was followed to isolate the liver and carcass fatty acids and count the radioactivity. Results were expressed in percent counts retained multiplied by 10^3.

From the data in the table, it is readily apparent that the fed hypothalamic obese mice incorporated considerably more acetate into fatty acid than the nonobese (approximately 4 times as much). Lipogenesis was greater both in the carcass (3 times) and in the liver (over 7 times). On the other hand, in the fasted state, total incorporations were not significantly different between obese and nonobese mice. Carcass lipogenesis was similar in obese and nonobese mice. There was some difference between liver incorporation in the two types of animals, of borderline significance ($p \leqslant 0{\cdot}05$) as had been observed in the hypothalamic obese rats. The significance of this slight residual difference, not demonstrated by isolated liver slices[4] is obscure, but is most probably related to the differences in liver weights between obese and nonobese animals. Actually, if liver incorporation is expressed on the basis of counts per gram, the counts in the obese mice are identical to those in the nonobese.

Incidentally, it is of interest to note that the livers of the obese mice used in the "fasting" experiment were larger than those of the group used in the "nonfasted" experiment. The former had been in a state of "dynamic" obesity (rapid weight gains), while the latter had gained weight more slowly. The animals of both groups were of similar over-all size. Furthermore, liver weight is very sensitive to the nutritional status and was most probably decreased somewhat by the 16 h fast. This finding would tend to indicate that the increase in liver weight encountered in various forms of experimental obesity[1] is probably related, at least in part, to the hyperphagia rather than to the excess weight as such.

The results presented here on lipogenesis confirm that the results previously obtained on hypothalamic hyperphagic rats are generally valid for hypothalamic hyperphagia in other species. They thus confirm the suggestion that this syndrome is of "regulatory" rather than "metabolic" nature.

J. Mayer and Claudine Y. Zighera

Department of Nutrition, Harvard School of Public Health, Boston, Mass., April 18, 1955.

Zusammenfassung

Bei Mäusen und Ratten mit hypothalamischer Fettsucht beobachtet man bei Fütterung *ad libitum* vermehrte Fettbildung, die zurückgeht, wenn man die Tiere hungern lässt. Diese Befunde lassen auf einen «regulatorischen» und nicht «metabolischen» Charakter dieser Fettsucht schliessen.

[1] R. G. French, C. Y. Zighera, and J. Mayer, Fed. Proc. *14*, 433 (1955). – J. Mayer, R. G. French, C. Y. Zighera, and R. J. Barrnett, Amer. J. Physiol. (in press, July 1955).

[2] J. Mayer, Physiol. Revs. *33*, 472 (1953).

[3] M. W. Bates, C. Zomzely, and J. Mayer, Amer. J. Physiol. *181*, 187 (1955).

[4] J. Mayer, N. C. Hagman, N. B. Marshall, and A. J. Stoops, Amer. J. Physiol. (in press, June 1955).

[1] J. Mayer, Proc. IIIrd Internat. Nutrition Congr., Voeding *16*, 62 (1955). – N. B. Marshall and J. Mayer (to be published).

Paléontologie et expérimentation

Contre les hypothèses voulant expliquer par des actions mécaniques la morphogénie dentaire, il était facile de trouver des arguments; en particulier, l'existence de dents de forme normale, bien qu'hétéropiques ou qu'implantées de travers, pouvait être invoquée. Des faits de même ordre ont pu être provoqués grâce à des cultures de tissus. Déjà, Glasstone[1], en explantant des germes de dents de Rat, avait prouvé que la formation, la forme et le nombre des cuspides n'étaient déterminés que par des facteurs endogènes[2]. Verne[3] a montré que des germes dentaires, prélevés sur des embryons, évoluaient avec

[1] A. H. Glasstone, Dent. Rec. *1938*.

[2] En 1940, A. G. Lapchinsky et A. A. Malinovsky[4] réussissaient également à obtenir des molaires ou des incisives, d'apparence normale, en prélevant des germes de rat ou de chien et en les insérant, soit dans une autre région des mâchoires, soit à la surface du fémur.

[3] J. Verne, Rev. odontol. *64*, 55 (1942).

[4] A. G. Lapchinsky et A. A. Malinovsky, C. r. Acad. Sci. U.R.S.S. *26*, 722 (1940); *29*, 268 (1940).

formation de cuspides normales en forme, nombre et arrangement. Cela démontrait que la morphogénèse des dents n'était pas réglée par des actions mécaniques; cela démontrait, de plus, le pouvoir inducteur, différenciateur, des adamantoblastes.

D'autre part, une observation de Petit et Budker montre que le système nerveux, qui, ailleurs, joue le rôle d'inducteur (expériences de Locatelli), peut influer sur la morphologie dentaire; elle est relative aux «dents cutanées» visibles sur la peau des embryons de certains sélaciens (*Carcharinus* etc.); ces dents sont des spicules différant, par leur forme et leur taille, de ceux qui constituent le revêtement du corps; or elles se trouvent exclusivement au niveaux de dépressions épithéliales dont le fond est occupé par un organe sensoriel.

Le rôle du système nerveux paraît ici comparable à celui qu'il joue aussi dans le cas des écailles de la ligne latérale des poissons.

Cela ne contredit pas ce fait indiqué par Rabaud[1], à savoir que la genèse et la localisation des zones de calcification sont liées à la répartition du réseau vasculaire (suivant un principe bien exposé par Leriche).

Tout cela montre l'importance des organisateurs, des inducteurs, en morphogénèse dentaire, ce qui permet d'interpréter de façon simple certains faits paléontologiques ou anatomiques[2].

On sait que les carnivores se distinguent non seulement par la forme de leurs carnassières mais encore par leur place dans la série dentaire; parmi les créodontes, les *Acreodi* (*Arctocyon, Pachyaena*) n'avaient pas de carnassières, les *Pseudocreodi* avaient des carnassières de formule $K = M_2/M_3$ (*Hyaenodon, Proviverra*) ou $K = M_1/M_2$ (*Oxyaena, Palaeonictis*) et les *Eucreodi* (*Viverravus, Miacis*) réalisaient déjà la disposition des carnivores actuels, $K = P_4/M_1$. Pour cette raison, on a dit que seuls les *Eucreodi* avaient pu donner naissance à ces derniers. Il paraît, au contraire, possible d'expliquer le glissement de la carnassière vers l'avant par une modification du champ d'action d'un inducteur. De même pourrait s'expliquer pourquoi la carnassière elle-même, qui typiquement est mi-coupante, mi-broyeuse, perd, dans certains cas, soit sa partie tranchante, soit sa partie tuberculée.

Les primates fournissent d'autres exemples. Chez les anthropomorphes, la prémolaire antérieure tend à ressembler tantôt à une canine, tantôt à une molaire. Au sein même d'une espèce, il y a des variantes, ainsi le jeune néanderthalien du Moustier a une canine supérieure qui tend à ressembler à une prémolaire et des prémolaires qui tendent à être molariformes. Ces exemples de convergences morphologiques entre dents voisines sont nombreux: canines incisiformes des lémuriens et des ruminants, canines prémolariformes des *Saghatherium*, molarisation des prémolaires de nombreux types. Les actions inductrices permettent d'envisager aisément la réversibilité de l'évolution de certains détails.

La possession de techniques récentes permettant des cultures d'organes[4], et non plus de simples cultures incoordonnées comme celles de Carrel, permet d'espérer la reproduction expérimentale de faits de cet ordre. Il est classique qu'il y a, chez le jeune veau, des germes des incisives supérieures qui n'aboutissent pas et manquent chez l'adulte, qu'il y a, chez les embryons de mystacocètes, des rudiments de dents, partiellement calcifiés, qui disparaissent avant la naissance, que, chez les édentés (*Orycteropus* etc.), il y a avortement des dents de lait, que celles-ci ne sont jamais fonctionnelles chez les insectivores et les chéiroptères, tandis que la seconde dentition avorte chez les odontocètes qui conservent toute la vie leurs dents lactéales. Il serait très intéressant si, par la culture de germes voués à l'atrophie, l'on reconnaissait qu'un tel facteur peut activer leur développement. D'autres essais seraient à faire en modifiant, soit *in vivo* l'induction nerveuse ou l'irrigation sanguine (dont on sait les relations avec la calcification), soit, *in vivo*, l'apport d'hormones (comme l'a fait, même *in utero*, Vera Dantchakoff).

On sait quel peut être le rôle inducteur d'une hormone, celle-ci pouvant être l'inducteur de sa propre glande sécrétrice (Wolff) ou inhiber le développement d'un organe (action de l'hormone mâle sur le canal de Müller). On sait, d'autre part leur rôle morphogénétique; l'influence des hormones mâles est responsable des caractères sexuels secondaires tels que la puissante canine des grands anthropomorphes.

Or il y a là une question capitale relativement aux origines de l'homme. Celui-ci peut-il ou non descendre d'un ancêtre à canines saillantes du type des anthropomorphes actuels. Il semble que, les hormones ne pouvant agir qu'à partir d'un certain seuil, une simple variation endocrinienne ait pu entraîner une évolution en sens inverse de celle qui avait abouti au type à grandes canines; autant que les données paléontologiques, très fragmentaires, du Fayoum peuvent être invoquées, les premiers anthropomorphes avaient des canines non saillantes.

Van Wagenen et Hurme[1], à l'aide de propionate de testostérone, ont avancé d'un an l'éruption de la canine d'un macaque; or, agissant à un stade différent, le même facteur pourrait fort bien entraîner un changement de taille.

D'autres espoirs sont permis. En effet, spontanément, sous l'influence de facteurs non encore analysés, les ébauches pentadactyles embryonnaires (palettes) des membres du cheval pouvent donner les doigts II et IV que possédait son ancêtre tridactyle (type *Hipparion* ou mieux *Protohippus*)[2]; on peut donc espérer obtenir systématiquement, grâce à un stimulant approprié, le développement de tels doigts. Et la remarque vaut pour tout organe dont l'ébauche embryonnaire est conservée. On n'oubliera, d'ailleurs, pas que, si parfois un membre tridactyle de type hipparion se développe, parfois aussi les phalanges ou métapodiaux surnuméraires ne correspondent nullement au type ancestral. Boas[3] (1917) a figuré des exemples où la polydactylie du cheval est absolument monstrueuse.

E. Patte

Institut de Géologie et d'Anthropologie préhistorique, Poitiers, le 7 juin 1955.

[1] Et. Rabaud, Rev. odontol. Sept. Oct. *49*, 611 (1937).

[2] Dans le cas des anomalies dentaires, Dieulafé et Herpin[3] observent qu'elles peuvent être soit isolées, soit paires et symétriques, soit même représentées au niveau des dents homologues des 2 mâchoires; ces auteurs ont expliqué le fait par une action simultanée sur plusieurs ébauches à un moment donné du développement; on peut imaginer, avec autant de vraisemblance, l'influence d'inducteurs.

[3] Dieulafé et Herpin, *Anatomie de la bouche et des dents* (Paris, Baillière 1928).

[4] Travaux du Professeur Etienne Wolff, de Strasbourg.

[1] M. Van Wagenen et G. Hurme, Proc. Soc. exper. Biol. Med. U.S.A. *73*, 296 (1950).

[2] Des faits analogues sont signalés chez les artiodactyles (mouton, porc).

[3] J. E. V. Boas, Zool. Jb., Abt. Anat. Ontogenie *40*, 49 (1917); Kgl. Veterinaer og Landbohøjkole, Aarskrift *1922*, 66.

Summary

Since the morphogenesis of teeth is ruled by organizers (inductors) the change of position of one of them could have brought about an evolution of the denture such as is found in the *Creodontia* and Carnivores. Modern techniques of organ cultivation will perhaps solve some other paleontological problems (reappearance of teeth whose germs miscarry, modification of the size of the canines, which would permit us to suppose a certain reversibility).

PRO EXPERIMENTIS

Sharpening of Knives for Ultramicrotomy

Ultrathin tissue sectioning for electron microscopy demands among other things a cutting edge of very high quality. The best edges are up to the present undoubtedly obtained by handpolishing razor blades[1], but this method has some definite drawbacks. In order to obtain better and more reproducible sharpening results we have investigated several kinds of knife-steel and, furthermore, worked out a special sharpening method.

The steel in ordinary microtome knives and razor blades is not particularly suitable for ultramicrotome knives. The reason for this is firstly the richness in very hard iron carbide particles which sometimes are wrenched out of the martensite during the sharpening and thus spoiling the edge. Secondly, the lack of corrosion resistance makes it impossible to use a knife for sectioning for more than a couple of hours.

Testing several kinds of steel we have found that a special steel containing 0·5 % carbon and 13 % chromium is very suitable for our purpose. The low carbon percentage makes it possible to obtain a steel composed mainly of martensite with very few or no carbide particles. By a special hardening process and by cautious tempering the steel is given a high hardness (about 800 Vicker units). The corrosion resistance is very good which is of vital importance. (This steel is produced by the Steel Company of Uddeholm.)

Fig. 1.

The knives of this special steel are sharpened by a lapping technique[2], carried out in two phases on a lapping machine. This consists of a cast iron plate fixed on a vertical axis and rotatable at variable speeds. In the first phase of lapping a special lapping disc of cast iron is used, the surface of which is extremely even and smooth and provided with circular grooves in order to prevent suction of the knife (Fig. 1). In the second phase of lapping this cast iron disc is exchanged for a mirror grade glass plate.

Fig. 2.

While sharpening the knife is mounted in a special fixture that always holds the knife in the same position. The fixture is provided with a perspex back that is resting on the lapping disc during the sharpening and so determines the bevel angle (see Fig. 1). For lapping against cast iron the fixture gives a bevel angle of 30°. While lapping against glass the knife is moved to a fixture the back of which is resting with one point on the glass and giving a bevel angle of 50° (Fig. 2.)

Before lapping against cast iron a thin layer of a paste, consisting of chromium oxide in oil is carefully rubbed into the lapping disc. The disc is rotating at about 40 r.p.m. The knife in its fixture is placed with the edge against the direction of rotation and moved to and fro in radial direction. The knife is turned over at intervals of about 15 s. The facet surfaces obtained have a high eveness and smoothness.

If the knife has big scratches on its facets or if it has not previously been used for ultramicrotomy we carry out an additional lapping against cast iron before the phase just described. We then use a lapping disc of the above kind and the same fixture (i.e. the bevel angle is 30°). To this disc we apply a paste consisting of carborundum which gives a higher rate of material removal but a coarser facet surface.

After having cleaned the knife in white spirits and mounted it in the second fixture the lapping against glass is carried out[1]. For this no abrasive is used, on the contrary it is carried out in a 3 % sodium bicarbonate solution containing one drop of detergent per 25 ml. The rotation velocity of the glass disc is about 10 r.p.m. No pressure is exercised on the knife during sharpening as the fixture is so balanced that its weight gives the adequate pressure.

Lapping against glass for 2–3 min gives facet surfaces of extreme eveness and smoothness and a continuous edge line that is perfectly straight and uniform. The width of these final facet surfaces is 10–15 μ, which is quite sufficient. (The inspection of the sharpening results is made in a darkfield microscope with a resolving power of about 0·7 μ and magnification of 700 times.)

[1] F. Sjöstrand, J. Cell. Comp. Physiol. *42*, 15 (1953).

[2] O. Hallén, Lab. Invest. (in press).

[1] "Lapping" is probably not the adequate term for this part of the sharpening since "lapping" signifies a removal of material and the glass phase implies very likely a smoothing out of the facets without any significant removal. However, the term "lapping" is used here for the sake of simplicity.

The swift production of the final facets is possible owing to the fact that the bevel angle is altered previous to the lapping against glass. Owing to this only a fraction of the cast iron lapped facets must be smoothed out. The pre-requisite for such an altering of the bevel angle is, on the other hand, that the cast iron lapped facets are very even.

Using the described steel and technique it is simple to sharpen knives of any kind, provided that the fixture is fitted to the knife. In our laboratory we use knives of the same size as ordinary microtome knives as well as of razor blade shape.

The most important advantages of our method seem to be:

(1) A cutting facet of a small but uniform width along the whole knife.

(2) The glass lapping is performed without any kind of abrasive (as no significant removal of material is required) and so scratches in the facets are avoided.

(3) Due to the rather extensive mechanization of the technique it requires no special dexterity or long practice and gives an almost 100% yield.

(4) The special, stainless steel is very suitable for the sharpening procedure and makes it possible to section with the same knife for several days without re-sharpening.

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Departments of Anatomy and Histology, University of Gothenburg, April 27, 1955.

Zusammenfassung

Die Verfasser beschreiben eine Methode zur Herstellung von Messerschneiden zum Ultramikrotomgebrauch. Das Material des Messers besteht aus rostfreiem Stahl, der aus fast reinem Martensit aufgebaut ist. Das Schärfen wird auf einer speziellen Läppmaschine ausgeführt und umfasst zwei Phasen. Die erste, das Läppen gegen Gusseisen, gibt dem Messer einen Schneidenwinkel von 30°. Die zweite und letzte Schärfungsphase wird mit Spiegelglas ausgeführt und gibt einen Schneidenwinkel von 50°.

PRO EXPERIMENTIS

Die Bestimmung von Dibenamin im biologischen Material

Für die Bestimmung von Dibenamin (N,N-Dibenzyl-β-chloräthylamin) und dibenaminähnliche Verbindungen benutzten wir bisher die von Brodie und Mitarbeitern[1] angegebene Methode, nach der Dibenaminalkohol (N,N-Dibenzyl-β-hydroxyäthylamin) mit Methylorange ein Molekülkomplex bildet, dessen Konzentration kolorimetrisch bestimmt werden kann. Dibenamin selbst geht keine Molekülverbindung mit Methylorange ein und muss deshalb durch alkalische Hydrolyse zum Alkohol umgesetzt werden. Abgesehen davon erweist sich das Verfahren als umständlich und nicht sehr empfindlich. Die ungenügende Genauigkeit dieser Methode dürfte im wesentlichen darauf zurückzuführen sein, dass einfache Azofarbstoffe eine zu geringe Neigung zur Adduktbildung aufweisen. Es wurde deshalb eine neue Nachweismethode für Dibenamin und dibenaminartige Verbindungen entwickelt, die nicht nur methodisch wesentlich einfacher ist, sondern auch genauere Bestimmungen zulässt.

Für die neue Methode verwenden wir Bromkresolpurpur, 5,5'-Dibromo-o-kresolsulfophthalein, als Adduktbildner. Dieser Farbindikator bildet mit vielen aromatischen Basen benzollösliche Farbstoffadditionsverbindungen, die analytisch folgendermassen angewendet werden: Die vom organischen Lösungsmittel (Benzol) aufgenommenen Addukte werden mit NaOH zersetzt. Die Base verbleibt im Lösungsmittel, der Farbstoff geht in die wässrige Phase und kann dort kolorimetrisch bestimmt werden.

Die zu untersuchende wässrige Dibenaminlösung (10 ml) wird in einem Scheidetrichter nach Zusatz von 5 ml Phosphatpuffer nach Sörensen (pH 5,2) und 5 ml Bromkresolpurpurlösung[1] mit 50 ml Benzol 2 min geschüttelt. Die wässrige Phase wird abgetrennt und nochmals mit 50 ml Benzol ausgeschüttelt. Die beiden Benzolauszüge werden vereint, filtriert und zweimal mit je 10 ml n/20 NaOH ausgeschüttelt. Die blau-violetten Natronlaugenextrakte werden vereinigt und mit n/20 NaOH nach Filtration auf 25 ml aufgefüllt. Mit dem Pulfrich-Photometer (Filter S 59[2], 2-cm-Küvette, Vergleichslösung *Aqua dest.*) erfolgt die Messung der Extinktion. Die Eichkurve entspricht im Bereich von 25 bis 400 γ pro 10 ml dem Lambert-Beerschen Gesetz.

Die Bildung der Farbstoffadditionsverbindung mit Bromkresolpurpur ist stark pH-abhängig. Die Kontrolle erfolgte mit der Jenaer Glaselektrode in Verbindung mit dem Nüsslein-Messverstärker (Typ 140).

Bestimmung von Dibenamin im Urin: Zur Einhaltung des optimalen pH-Wertes von 5,2 wird zweckmässig der Urin mit verdünnter Phosphorsäure auf ein pH von etwa 5,3 gebracht, damit die Einstellung durch den Phosphatpuffer gesichert ist. Die Bestimmung erfolgt, wie oben beschrieben. Die Blindwerte einer Reihe normaler Urine lagen bei dem eingehaltenen optimalen pH-Wert sehr niedrig und konstant.

Bestimmung von Dibenamin im Serum: 1 ml Schweineserum wird mit 1–2 Tropfen 1 n HCl versetzt und 14 ml Phosphatpuffer zugegeben (pH 5,2). Bei dieser Bestimmung wird 4 min mit 2mal 50 ml Benzol ausgeschüttelt.

Bestimmung von Dibenaminalkohol: Die Additionsverbindung entsteht im stärker sauren pH. Die wässrige Dibenaminlösung bzw. der dibenaminhaltige Urin werden durch Alkalisierung und zweistündige Erwärmung auf 40° hydrolysiert. Danach wird mit 2 n HCl auf pH 4,5 eingestellt und wie oben ausgeschüttelt. Als Vergleichslösung dient *Aqua dest.* bzw. Leerurin.

Die neue Methode ermöglicht die Bestimmung von Dibenamin (Hydrochlorid) mit einer Empfindlichkeit von 2,5 γ/cm³ Urin bzw. von Dibenaminalkohol 5,0 γ/cm³ Urin.

Neben der grösseren Empfindlichkeit hat sich die Bromkresolpurpurmethode als wesentlich einfacher erwiesen. Sie hat sich für die Bestimmung von Dibenamin und dibenaminartigen Verbindungen in Urin und Serum für Reihenuntersuchungen bewährt.

H. Hofmann, K.-H. Boltze und D. Weyland

Pharmakologisches Institut der Friedrich-Schiller-Universität Jena, den 15. Mai 1955.

[1] B. B. Brodie und Mitarbeiter, J. Pharmacol. exp. Ther. *106*, 166 (1952).

[1] Zur Bereitung der Bromkresolpurpur-Farblösung löst man 0,2 g Bromkresolpurpur in 50 cm³ *Aqua dest.* unter Zusatz von 3,2 cm³ n/10 NaOH und füllt mit *Aqua dest.* auf 250 cm³ auf.

[2] Für Dibenamin-Hydrochlorid wurde ein Absorptionsmaximum (100 γ/10 ml *Aqua dest.*, Schichtdicke $s = 2$ cm; Filter $S = 59$) von 5820 Å bestimmt.

Summary

In order to identify the presence of dibenamine and dibenamine-like compounds in biological substances (urine, sera) bromine cresol purple was used as a formative element in the adduction of dyestuff (i.e. as "Farbstoffadduktbildner"). This method permits a more simplified and more sensitive process of work than that suggested by BRODIE and his collaborators.

PRO LABORATORIO

A Generator for Homogeneous Aerosols

A simple generator has been constructed, giving a continuous supply of between 3·7 to 10 lit/min of homogeneous aerosol. Like other generators[1], it is based on successive evaporation and recondensation of the aerosol-producing substance. This generator uses a nebulizer for primary formation of an aerosol, the aerosol being subsequently evaporated on passing through a heated tube. On recondensation of the vapour, an homogeneous aerosol is produced. This procedure eliminates the need for prolonged heating of the substance in bulk and permits high mass-concentrations to be obtained.

Construction (see Figure 1). The generator consists of A) a nebulizer, B) an evaporator, and C) a recondensing chimney.

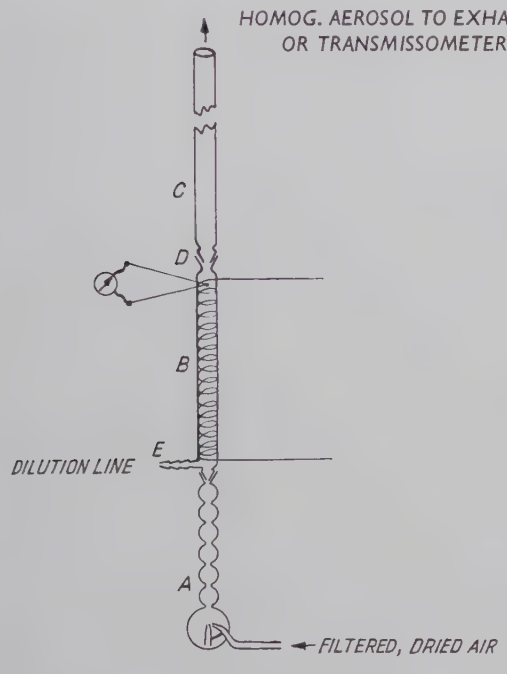


Fig. 1.

(A) *is a Dautrebande nebulizer*[2], giving an aerosol with a sharp upper limit of radius (~ 1·2 micron) and standard deviation of 20–30 %. The model used in the present investigation produces at least 3·7 l/min of a heterogeneous aerosol, with a particle concentration of 5×10^4–10^8 per cm³. Above the nebulizer is an inlet E for air, as it is occasionally necessary to dilute the aerosol in order to obtain optimum results (see Table I).

Table I

Air through nebulizer (*in l/min*)	Air for dilution (*in l/min*)	Mean radius (*in microns*)	Standard deviation	Number concentration (*in particles/cm³*)
3·7	2·0	0·508	7½%	$8{\cdot}5 \times 10^4$
3·7	2·0	0·462	9 %	$8{\cdot}5 \times 10^4$
5·2	2·3	0·539	8½%	6×10^5
5·2	2·3	0·554	9½%	6×10^5
4·7	4·3	0·608	9½%	4×10^5
4·7	4·3	0·540	9½%	$3{\cdot}5 \times 10^5$

(B) *The evaporator* consists of a pyrex tube 25 cm long, with internal diameter of about 20 mm. It is heated by a resistance wire wound on the outside. The wire is connected to a variable transformer, enabling changes in the temperature to be made. The optimum temperature, measured by a copper-constantan thermocouple D, was found to be about 180°C for dioctyl phthalate (D.O.P.).

C) *The chimney* consists of a glass tube, 120 cm long and with internal diameter of about 25 mm.

The aerosol produced in the nebulizer is evaporated completely (no discernible Tyndall effect) in the evaporator and recondensed in the chimney, without addition of condensation nuclei. Dried and filtered air is used throughout.

Homogeneous aerosols of diethyl, dibutyl and dioctyl phthalates, stearic acid, oleic acid, tri-o-cresyl phosphate and menthol were prepared. The aerosols showed distinct higher order Tyndall spectra.

By way of example, some results with dioctyl phthalate are given in Table I. Table II summarizes the experiments with the same substance but using a slightly modified generator, which gave an aerosol of at least equal homogeneity, without the need for dilution. An elongated bulb of about 200 ml capacity, heated in a similar manner to the evaporator, was inserted between the evaporator and the chimney.

Table II

Air through nebulizer (*in l/min*)	Air for dilution (*in l/min*)	Mean radius (*in microns*)	Standard deviation	Number concentration (*in particles/cm³*)
4·7	–	0·553	9½%	$4{\cdot}5 \times 10^5$
5·7	–	0·573	8½%	$1{\cdot}5 \times 10^6$
6·7	–	0·557	9½%	2×10^6
7·7	–	0·579	9 %	4×10^6
8·7	–	0·558	9½%	$\sim 10^7$

The particle radii were measured in a convection-free ultramicroscope cell. The concentration of the particles was determined in a BECKMAN spectrophotometer[1], the aerosol streaming through the quartz gas-cells, or preferably in an especially constructed transmissometer (optical length: 50 cm) using the 5460 A line of an A-H-4 lamp. The concentrations were calculated by the usual method[2].

[1] D. SINCLAIR and V. K. LAMER, Chem. Rev. *44*, 245 (1949).

[2] L. DAUTREBANDE, Z. Aerosol-Forschung (Okt. 1953); p. 585 ff.; "L'Aerosologie" (Paris, 1951), pp. 6–21.

[1] E. C. Y. INN, J. Coll. Sci. *6*, 368 (1951).

[2] V. K. LAMER, E. C. Y. INN, and I. WILSON, J. Coll. Sci. *5*, 482 (1950).

This generator, which has been most successfully used in a number of investigations, has several advantages over the known types: it is of simple construction and operation, and requires only a few minutes to reach stationary conditions. It produces larger quantities of aerosol than other apparatus and higher particle concentrations (up to 10 l/min of homogeneous aerosol and 10^7 particles per cm^3). Even substances which tend to decompose on prolonged heating can easily be converted into aerosols (e.g. D.O.P.), and the results are, within limits, reproducible (see Table I).

This investigation was sponsored by the Scientific Department, Israeli Ministry of Defence, and is published with its permission. We wish to thank Mr. R. Aldor for carrying out the measurements and calculations of the particle radii.

E. Rapaport and S. E. Weinstock

Ministry of Defence, Scientific Department, Tel-Aviv, Israel, May 28, 1955.

Zusammenfassung

Es wird ein einfacher neuer Generator für homogene Aerosole beschrieben, welcher grössere Mengen und höhere Konzentrationen, als bisher bekannt sind, liefert. Der Apparat erzeugt homogene Aerosole auch von Substanzen, welche nicht hitzebeständig sind.

Nouveaux livres - Buchbesprechungen - Recensioni - Reviews

The Design and Analysis of Industrial Experiments

By Owen L. Davies

637 pages
(Oliver and Boyd, Edinburgh, 1954)
(63/–)

Dieses Buch, von einem Team von Chemikern und Statistikern geschrieben, wendet sich in erster Linie an den Forscher in der chemischen und pharmazeutischen Industrie. Planung, Durchführung und Auswertung von Experimenten werden darin vom Standpunkt der statistischen Methoden aus dargelegt, wobei der Nachdruck auf der Planung der Versuche unter besonderer Berücksichtigung ihrer Wirtschaftlichkeit liegt.

Die Ergebnisse chemischer Versuche pflegen häufig in aufeinanderfolgenden Serien anzufallen, und es ist dann wichtig, die ganze Versuchsfolge so anzuordnen, dass Ergebnisse früherer Versuche bei der Bestimmung von Richtung und eventuell Umfang weiterer Versuche möglichst erschöpfend berücksichtigt werden. Im Anschluss an die Darstellung statistischer Tests im allgemeinen, die hauptsächlich vom Standpunkte der Versuchsplanung und unter Berücksichtigung der mit den Tests verbundenen Risiken erfolgt, wird daher ausführlich auf die sogenannten Folgetests eingegangen.

Unter den eigentlichen Versuchsplänen werden zunächst zufällige Anordnungen in Blöcken, lateinische und griechisch-lateinische Quadrate und die verschiedenen Varianten der Anordnung in unvollständigen Blökken behandelt. Eine besonders eingehende Darstellung wird den Versuchen mit mehreren Faktoren, dem vollständigen und teilweisen Vermengen von Wechselwirkungen mit Unterschieden zwischen den Blöcken und den teilweise wiederholten Versuchen zuteil.

Die Versuche mit mehreren Faktoren leiten über zu den Verfahren, welche die Bestimmung der optimalen Arbeitsbedingungen in chemischen Prozessen zum Gegenstand haben. Diese Methoden zählen zu den neuesten der Statistik und dürften im Rahmen eines Lehr- oder Handbuches bisher kaum dargestellt worden sein. Besonders begrüssen wird es der Chemiker, dass er hier auch gleich die mathematischen Hilfsmittel und, im Hinblick auf die Anwendungen, die entsprechenden numerischen Methoden, deren Verwendung in diesem Zusammenhang nicht ganz umgangen werden kann, auseinandergesetzt findet.

Es gibt zahlreiche quantitativ-chemische Fragen, zu deren Lösung die Anwendung statistischer Verfahren den einzig methodisch einwandfreien und logisch sauberen Weg darstellt. Die statistische Seite mancher chemisch-technologischer Probleme zu erkennen, die Fragestellung entsprechend zu formulieren und selber die Methoden anzuwenden, dazu möchte das Buch den Chemiker in der Industrie anleiten. In der Stoffauswahl sind die Verfasser bestrebt, das für den Chemiker Wichtige in den Vordergrund zu stellen. Anderseits ist die Darstellung genügend allgemein gehalten, dass sie auch Ingenieure und Techniker verwandter Disziplinen interessieren dürfte. Die wesentlichen Ideen werden klar zum Ausdruck gebracht und, um die Entwicklung prinzipieller Fragen nicht zu unterbrechen, findet die Begründung einzelner Verfahren und die Diskussion spezieller Gegenstände in den Anhängen zu den einzelnen Kapiteln Platz, welche etwa den dritten Teil des Buches einnehmen.

Das Buch ist klar und leichtfasslich geschrieben. Die mathematischen Kenntnisse, die es voraussetzt, sind recht bescheiden und die Darstellung bleibt immer anschaulich und direkt. Wertvoll sind vor allem die etwa fünfzig ausführlichen Beispiele, die dem Gebiet der chemischen Technologie und verwandten Gebieten entstammen. Ein Glossar statistischer Fachausdrücke und zahlreiche Tabellen samt Erläuterungen sowie ein ausführliches Sachregister beschliessen dieses Werk, das eine wertvolle Bereicherung nicht nur der statistischen, sondern auch der technischen Literatur darstellt. Wir zweifeln nicht daran, dass es die Anwendung statistischer Methoden in der Industrie erheblich fördern wird.

A. Linder

Informations - Informationen - Informazioni - Notes

STUDIORUM PROGRESSUS

Über Synthesen in der Aldosteron-Reihe I[1]. Totalsynthese des racemischen Aldosterons[2]

Von J. Schmidlin, G. Anner, J.-R. Billeter und A. Wettstein[3], Basel

Seit der Konstitutionsermittlung[4] des aus Nebennieren vor etwa zwei Jahren erstmals isolierten[5] hochwirksamen Steroidhormons Aldosteron war es unser Bestreben, diese aus der endokrinen Drüse nur in äusserst beschränkten Mengen extrahierbare Substanz durch Synthese einer eingehenderen biologischen Untersuchung zugänglich zu machen. Im folgenden berichten wir in Kürze[6] über erfolgreich verlaufene Versuche, das dem natürlichen Wirkstoff entsprechende Racemat (XXIV) auf totalsynthetischem Wege zu bereiten.

Ausgegangen wurde vom *d,l*-Δ^{8a}-4bβ-Methyl-7-äthylendioxy-4aα,10aβ-dodecahydrophenanthren-4β-ol-1-on (I), welches nach Sarett und Mitarbeitern[7] aus Propionsäure über 1,3,3-Triäthoxypentan und 3-Äthoxy-pentadien durch Kondensation mit Benzochinon und später Methylvinylketon in etwa 10 Stufen zugänglich ist. Seine Carbäthoxylierung mit Diäthylcarbonat in Stellung 2 führte unter gleichzeitiger Veresterung der 4β-Hydroxylgruppe zum β-Ketoester II (Eigenschaften siehe Tabelle). Alkylierung von II mit Methallyljodid[8] ergab vorwiegend das 2α-Methallyl-Derivat III. Die α-Konfiguration des neu eingeführten Substituenten folgt aus der glatt verlaufenden Überführung von III in das β-orientierte γ-Lacton der Formel VI. Hiefür wurde der alkaliempfindliche β-Ketoester III zunächst durch Reduktion mittels $NaBH_4$ in den Oxyester IV übergeführt. Die energische Behandlung mit Kaliumhydroxyd in siedendem Äthanol lieferte dann unter Hydrolyse der 2- und 4-ständigen Estergruppen die Dioxysäure V, die äusserst leicht, schon beim Erwärmen mit Benzol, in das Oxylacton VI überging. Die Dehydrierung der reaktionsträgen Oxygruppe in VI mit Chromtrioxyd in Pyridin[1] gelang erst bei erhöhter Temperatur. Das so erhältliche Ketolacton VII, das *d,l*-Δ^{8a}-4bβ-Methyl-2α-methallyl-1-oxo-4β-oxy-7-äthylendioxy-4aα,10aβ-dodecahydrophenanthren-2β-carbonsäure-lacton-(2$\rightarrow$4) stellte, wie aus den folgenden Umsetzungen hervorgeht, ein geeignetes Zwischenprodukt zur Angliederung des Ringes D dar.

Die Einführung der für den Aufbau des Fünfrings D noch fehlenden zwei Kohlenstoffatome erfolgte durch Umsetzung des Ketolactons VII mit Äthoxy-äthinyl-magnesiumbromid[2]. Dabei blieb die Lactongruppe unangegriffen und es entstand das tertiäre Carbinol VIII. Bei der Umformung der in 1- und 2-Stellung befindlichen Seitenketten unter Bildung zum Ringschluss befähigter Gruppen erwies es sich als vorteilhaft, vorerst den Methallylrest abzuwandeln, womit die Schwierigkeit einer selektiven Hydrierung der konjugierten Doppelbindung neben der endständigen Doppelbindung in der später zu durchlaufenden Stufe des α,β-ungesättigten Aldehyds (analog XII) entfiel. Durch Einwirkung von einem Äquivalent Osmiumtetroxyd in Gegenwart von Pyridin und reduktive Spaltung des Osmiumsäureesters bei pH = ca. 8,5 gelang es, das Carbinol VIII unter Erhaltung der leicht einer Retroaldolkondensation unterliegenden Zentren 1 und 2 in ein Gemisch der epimeren Glykole IX überzuführen.

Da Äthoxyäthinyl-carbinole in Gegenwart von Säure leicht Wasser anlagern, wurde das Epimerengemisch IX mit Vorteil vor dem Seitenkettenabbau an Palladium-Calciumcarbonat-Katalysator in Pyridinlösung selektiv zur Vinylstufe (X) hydriert. Die Oxydation von X mit Perjodsäure lieferte dann die einheitliche Verbindung XI, die eine Acetonyl- und eine Äthoxy-vinyl-Seitenkette aufweist. Die letztere erwies sich gegenüber Mineralsäuren als ungewöhnlich resistent. Erzwang man ihre Umlagerung durch energische Säureeinwirkung, so wurde gleichzeitig die bei der späteren selektiven Hydrierung noch notwendige Ketalgruppe gespalten. Die Überführung in XII unter vollständiger Erhaltung der Ketalgruppierung gelang jedoch glatt mit Phosphortribromid oder Thionylchlorid in wasserfreien Lösungsmitteln. Der α,β-ungesättigte Aldehyd stellt offenbar ein Gemisch *cis-trans*-isomerer Formen dar. Die katalytische Hydrierung von XII an Palladiumkohle kam nach Absättigung der konjugierten Doppelbindung zum Stillstand; neben einer untergeordneten Menge der in 1-Stellung epimeren Verbindung wurde dabei der gesättigte Aldehyd XIII erhalten, der, wie die folgenden Umsetzungen zeigten, ein α-ständiges Wasserstoffatom am neu entstandenen Asymmetriezentrum 1 aufweist. Cyclisierung von XIII zum Beispiel mittels Benzoesäure und Triäthylamin in siedendem Xylol[3] ergab das *d,l*-$\Delta^{5;16}$-3-Äthylendioxy-11β-oxy-20-oxo-pregnadien-18-säure-lacton-(18$\rightarrow$11) (XIV). Durch Hydrierung an Palladiumkohle-Katalysator ging es, unter Absättigung der konjugierten Dop-

[1] 132. Mitteilung «Über Steroide». Wurde am 22. Juli 1955 anlässlich des XIV. Internat. Kongresses für reine und angewandte Chemie in Zürich vorgetragen. 131. Mitteilung vgl. E. Vischer, Ch. Meystre und A. Wettstein, Helv. chim. Acta *38*, 835 (1955).

[2] Diese Arbeit bildet einen Teil umfangreicher Untersuchungen, an denen ausser den Laboratorien der CIBA AG., Basel, die Anstalt für Organische Chemie der Universität Basel (T. Reichstein, A. Lardon, O. Schindler und H. P. Uehlinger), die Forschungslaboratorien der N. V. Organon, Oss (D. A. van Dorp und S. Szpilfogel), sowie das Organisch-chemische Laboratorium der ETH., Zürich (V. Prelog, A. Eschenmoser, O. Jeger und H. Heusser) massgebend beteiligt sind. Über weitere Ergebnisse werden diese Forscher später berichten.

[3] Wissenschaftliche Laboratorien der CIBA AG., Basel, Pharmazeutische Abteilung. Bei der Redaktion eingegangen: 20. Juli 1955.

[4] S. A. Simpson, J. F. Tait, A. Wettstein, R. Neher, J. v. Euw, O. Schindler und T. Reichstein, Exper. *10*, 132 (1954); Helv. chim. Acta *37*, 1200 (1954).

[5] S. A. Simpson, J. F. Tait, A. Wettstein, R. Neher, J. v. Euw und T. Reichstein, Exper. *9*, 333 (1953); dieselben mit O. Schindler, Helv. chim. Acta *37*, 1163 (1954). – Vgl. auch R. Mattox, H. L. Mason und A. Albert, Proc. Staff Meet. Mayo Clinic *28*, 569 (1953); dieselben mit C. F. Code, J. Amer. Chem. Soc. *75*, 4869 (1953). – R. E. Harman, E. A. Ham, J. DeYoung, N. G. Brink und L. H. Sarett, J. Amer. Chem. Soc. *76*, 5035 (1954).

[6] Eine ausführliche Arbeit folgt in Helv. chim. Acta.

[7] G. I. Poos, G. E. Arth, R. E. Beyler und L. H. Sarett, J. Amer. Chem. Soc. *75*, 422 (1953), und frühere Veröffentlichungen.

[8] Vgl. L. H. Sarett, W. F. Johns, R. E. Beyler, R. M. Lukes, G. I. Poos und G. E. Arth, J. Amer. Chem. Soc. *75*, 2112 (1953).

[1] Zur Verwendung dieses Reagens siehe G. I. Poos, G. E. Arth, R. E. Beyler und L. H. Sarett, J. Amer. Chem. Soc. *75*, 422 (1953).

[2] J. F. Arens und D. A. van Dorp, Nature *160*, 189 (1947). – G. E. Arth, G. I. Poos, R. M. Lukes, F. M. Robinson, W. F. Johns, M. Feurer und L. H. Sarett, J. Amer. Chem. Soc. *76*, 1715 (1954).

[3] Vgl. zum Beispiel P. Wieland, H. Ueberwasser, G. Anner und K. Miescher, Helv. chim. Acta *36*, 376 (1953).

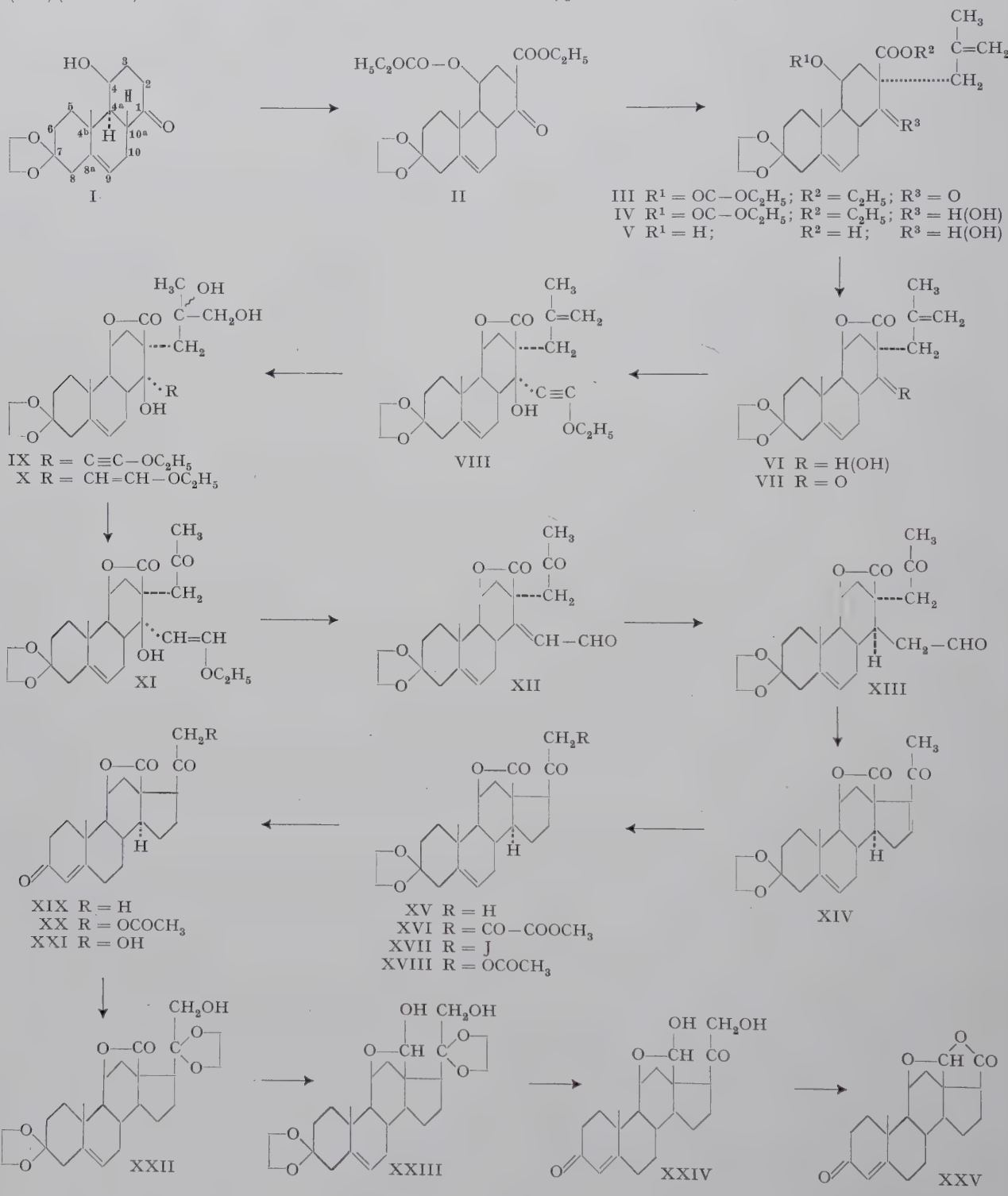
pelbindung in 16,17-Stellung, in das Pregnen-ketal XV über, das bei der Ketalspaltung das Progesteron-Derivat XIX lieferte.

Die dem Racemat XIX entsprechende optisch aktive Verbindung ist inzwischen von T. Reichstein und Mitarbeitern[1] aus natürlichem Aldosteron hergestellt worden. Beide Stoffe ergaben, in Methylenchlorid-Lösung untersucht, völlig übereinstimmende IR.-Spektren (siehe Abb. 2 in Publikation[1]). Damit war schon hier eine Bestätigung für den strukturell und sterisch richtigen Verlauf unserer Synthese gegeben.

Zur Hydroxylierung in 21-Stellung wurde das Methylketon XV durch Kondensation mit Oxalsäure-dimethylester[1] in die β-Dicarbonylverbindung XVI übergeführt, das Natrium-enolat der letzteren mit Jod umgesetzt, der 21-Jod-21-oxalosäureester zur Vermeidung einer Öffnung des Lactonringes mit Natriummethylat in wasserfreiem

[1] J. v. Euw, R. Neher und T. Reichstein, Helv. chim. Acta *38* (1955) (im Druck).

[1] H. Ruschig, Angew. Chem. *60A*, 247 (1952); Chem. Ber. *88*, 878 (1955). – G. I. Poos, R. M. Lukes, G. E. Arth und L. H. Sarett, J. Amer. Chem. Soc. *76*, 5031 (1954).



Nr.[a]	Smp.	Brutto-formel	Zusammensetzung[b]		UV.-Absorption[c]		IR.-Absorption[d]					
			% C	% H	λ_{max}	ε_{max}	—OH	–C≡C–	>C=O		>C=C<	Weitere charakteristische Banden
II	115–116°	$C_{23}H_{32}O_8$	63,10 (63,28)	7,46 (7,39)	256	12000			5,74		6,17	6,04 (Chelat) 7,28 8,35 9,72
III	99,5–101°	$C_{27}H_{38}O_8$	65,95 (66,10)	7,84 (7,81)	220–350	~0			5,72	5,82	6,07	7,28 9,13 9,75 10,56
IV	92–94,5° 99–101°	$C_{27}H_{40}O_8$	65,64 (65,83)	8,01 (8,19)	220–350	~0	2,84		5,74	5,86	6,08	7,30 8,87 9,16 9,54 10,47 11,81
VI	246–249°	$C_{22}H_{30}O_5$	70,34 (70,56)	8,18 (8,08)	—	—	2,80		5,65		6,07	7,33 9,11 9,50 10,04 10,57 10,75
VII	184–185°	$C_{22}H_{28}O_5$	70,79 (70,94)	7,58 (7,58)	—	—			5,61	5,80	6,06	7,37 7,60 8,37 9,08 9,76 10,04
VIII	138–140° (Zers.)	$C_{26}H_{34}O_6$	70,44 (70,56)	7,93 (7,74)	240–350	~0	2,77 2,84	4,42	5,62		6,06	7,32 8,50 8,76 10,00 10,54 10,75
IX	127–153° (Zers.)	$C_{26}H_{36}O_8$	—	—	—	—	2,75 2,98	4,42	5,64			7,32 8,78 9,99 10,55 11,52 12,05
X	148–151°	$C_{26}H_{38}O_8$	—	—	—	—	2,84		5,65		5,97	7,31 8,48 9,13 9,72 10,06 11,50
XI	122–124°	$C_{25}H_{34}O_7$	67,28 (67,24)	7,87 (7,68)	240–350	~0	2,84		5,64	5,81	5,98	7,33 8,45 8,74 9,13 10,00 11,51
XII	186,5–203° (Zers.)	$C_{23}H_{28}O_6$	68,77 (68,98)	7,10 (7,05)	242	7800			5,61 5,97	5,78	6,18	7,36 8,29 8,79 9,09 9,32 10,54
XIII	193–204,5° (Zers.)	$C_{24}H_{34}O_5$	—	—	220–350	~0			5,64	5,80		7,32 9,08 9,75 10,54 10,78
XIV	241,5–247°	$C_{23}H_{28}O_5$	71,75 (71,85)	7,21 (7,34)	237	8000			5,65	5,97	6,23	7,38 8,52 8,81 9,09 9,76 10,56
XV	194,5–215°	$C_{23}H_{30}O_5$	—	—	220–350	~0			5,64	5,84		7,36 8,23 9,08 9,76 10,56 10,80
XVI	192–196,5°; 198,5–202° (Zers.)	$C_{26}H_{32}O_8$	66,09 (66,08)	7,02 (6,83)	—	—			5,63	5,71		6,08/6,25 (Enol) 7,33 9,08 10,10
XVIII	265–268°	$C_{25}H_{32}O_7$	—	—	—	—	—	—		—	—	—
XIX	218–220°	$C_{21}H_{26}O_4$	73,44 (73,66)	7,92 (7,66)	238	14300			5,63 5,97	5,83	6,16	7,34 8,59 9,09 10,11 10,58 11,55
XX	244,5–247°	$C_{23}H_{28}O_6$	68,82 (68,98)	7,16 (7,05)	238	17400		e)	5,64 5,77	5,70 5,98	6,16	8,57 8,88 9,08 10,23 10,58 10,67
XXI	233–236° (Zers.)	$C_{21}H_{26}O_5$	69,90 (70,37)	7,32 (7,31)	—	—	2,83		5,62 5,95	5,82	6,15	7,32 8,55 8,85 9,07 9,30 10,65
XXII	261–264° (Zers.)	$C_{25}H_{34}O_7$	67,04 (67,24)	7,67 (7,68)	—	—	2,75		5,63			7,31 8,21 8,81 9,09 10,10 10,55
XXIII		$C_{25}H_{36}O_7$	—	—	—	—	2,76 2,83					9,06 9,21 9,49 9,68 10,54
XXIV	154°/183-185°	$C_{21}H_{28}O_5 \cdot H_2O$	66,62 (66,64)	8,14 (7,99)	—	—	2,68 2,77 2,88	e)	5,85	5,97	6,16	6,89 7,86 9,36 9,60 9,95 10,16 10,42 11,20 11,55
XXV	298–300°	$C_{20}H_{24}O_4$	—	—	—	—		e)	5,62	5,98	6,17	9,13 9,27 9,45 10,26 10,44

[a] Vgl. Formelübersicht. [b] Die berechneten Werte sind in Klammern angeführt. [c] Lösungsmittel durchweg 96-proz. Äthanol; Angabe der Wellenlängen in mμ. [d] Aufgenommen mit einem Perkin-Elmer-Spektrophotometer, Modell 21, mit NaCl-Prisma; Resolution 4, Response 1/1, Speed 2 min/μ, Suppression 1. Lösungsmittel, wo nichts anderes vermerkt, Methylenchlorid. [e] Lösungsmittel: Chloroform.

Methanol der Säurespaltung unterworfen und das entstandene Jod-keton XVII schliesslich mit Kaliumacetat in Aceton behandelt. Aus dem so bereiteten Ketolacetat XVIII gewann man durch Erwärmen mit 50-proz. Essigsäure das *d,l*-Δ^4-3,20-Dioxo-11β-oxy-21-acetoxy-pregnen-18-säure-lacton-(18→11) der Formel XX. Der Vergleich des in Chloroform-Lösung aufgenommenen IR.-Spektrums des racemischen Lactons XX mit demjenigen der aus Aldosteron erhaltenen optisch aktiven Verbindung[1] ergab Identität bis in alle Einzelheiten hinsichtlich Lage und relativer Intensität der Banden (siehe Abb. 1). Dies stellt einen neuen Beweis für die Übereinstimmung der synthetischen mit der natürlichen Reihe dar.

Zur Umwandlung von XX in das racemische Aldosteron blieb, ausser Hydrolyse, formal nurmehr die Reduktion der Lactongruppe in die Hemiacetalgruppe durchzuführen. In Übereinstimmung mit Vorversuchen an der tricyclischen Verbindung VI liess sich hiefür Lithiumaluminiumhydrid verwenden. Das aus Verbindung XVIII oder XX durch Hydrolyse mit Perchlorsäure unter Erhaltung der Lactongruppe leicht zugäng-

[1] S. A. Simpson, J. F. Tait, A. Wettstein, R. Neher, J. v. Euw, O. Schindler und T. Reichstein, Exper. *10*, 132 (1954); Helv. chim. Acta *37*, 1200 (1954).

liche 21-Oxy-diketon XXI[1] wurde vorerst zum Schutz der beiden Ketogruppen mit Glykol in Gegenwart von p-Toluolsulfonsäure[2] in das Diketal XXII umgewandelt. Die Einwirkung von Lithiumaluminiumhydrid auf XXII führte zum Cyclo-hemiacetal XXIII. Letzteres lieferte bei der Ketalspaltung, zum Beispiel mittels Salzsäure in Tetrahydrofuran, *d,l*-Aldosteron (XXIV).

Zur weiteren Charakterisierung wurde XXIV durch Oxydation mit Perjodsäure in das hochschmelzende, schwerlösliche und sublimierbare Lacton XXV übergeführt. Dieses wies bei der Papierchromatographie und im IR.-Spektrum Eigenschaften auf, die mit denjenigen des entsprechenden Derivates von natürlichem Aldosteron[1] identisch waren.

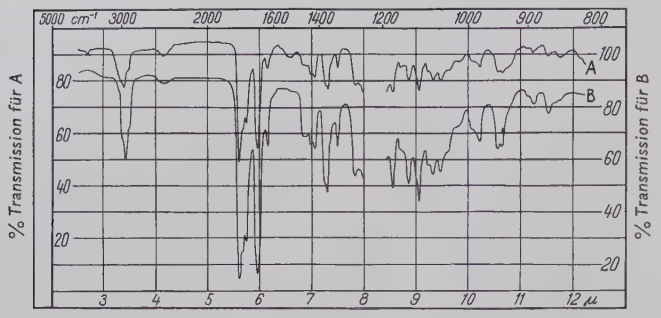


Abb. 1. IR.-Absorptionsspektren in $CHCl_3$, kompensiert mit reinem $CHCl_3$, Schichtdicke 0,2 mm[3]. *A*: *d*-Lacton aus Aldosteron-21-monoacetat (Reichsteins Verbindung Nr. 875)[4]. *B*: Totalsynthetisches *d,l*-Lacton XX.

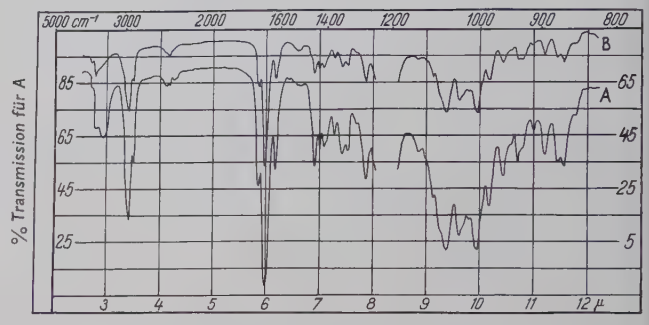


Abb. 2. IR.-Absorptionsspektren in $CHCl_3$, kompensiert mit reinem $CHCl_3$, Schichtdicke 0,2 mm[2], Mikrozelle. *A*: *d*-Aldosteron[3]. *B*: Totalsynthetisches *d,l*-Aldosteron (XXIV).

Das so totalsynthetisch erhaltene, racemische Aldosteron zeigte bei der Papierchromatographie in verschiedenen Lösungsmittelsystemen, in den untersuchten Farbreaktionen und im IR.-Spektrum völlige Übereinstimmung mit dem natürlichen, rechtsdrehenden Aldosteron aus Nebennieren (siehe Abb. 2[5]). Im modifizierten Kagawa-Test geprüft[6], erwies es sich erwartungsgemäss als etwa halb so wirksam wie *d*-Aldosteron bezüglich Na-Retention und K-Ausscheidung.

Dr. R. Neher danken wir für die papierchromatographischen Versuche, Dr. E. Ganz für die Aufnahme der IR.-Spektren und Dr. H. Gysel für die Elementaranalysen. Dr. K. Heusler, Dr. H. Ueberwasser und Dr. P. Wieland sind wir für Überarbeitung der Herstellungsmethode von I, Dr. P. Müller, Dr. M. Oberlin und Dr. F. Steinmann für die Bereitung grösserer Mengen von I und VII dankbar.

Prof. R. Meier und Dr. P. A. Desaulles verdanken wir die biologische Testierung von XXIV.

Summary

d,l-Aldosterone (XXIV) has been prepared by total synthesis, involving more than 20 new steps, from the tricyclic compound I.

[1] Eine analoge Reaktionsfolge, nämlich die Rückverwandlung der aus natürlichem Aldosteron hergestellten optisch aktiven Verbindung XXI in *d*-Aldosteron haben J. v. Euw, R. Neher und T. Reichstein [Helv. chim. Acta *38* (1955), im Druck] durchgeführt.

[2] Zur Methode vgl. W. S. Allen, S. Bernstein und R. Littell, J. Amer. Chem. Soc. *76*, 6116 (1954).

[3] Vgl. die methodischen Angaben bei der Tabelle.

[4] S. A. Simpson, J. F. Tait, A. Wettstein, R. Neher, J. v. Euw, O. Schindler und T. Reichstein, Helv. chim. Acta *37*, 1203, 1215 (1954).

[5] Der kleine Unterschied im Bereich der Hydroxylbanden erklärt sich lediglich durch die abweichende Konzentration. Siehe dazu [4].

[6] C. M. Kagawa, E. G. Shipley und R. K. Meyer, Proc. Soc. exper. Biol. Med. *80*, 281 (1952). – P. A. Desaulles, J. Tripod und W. Schuler, Schweiz. med. Wschr. *83*, 1088 (1953).

[1] S. A. Simpson, J. F. Tait, A. Wettstein, R. Neher, J. v. Euw, O. Schindler und T. Reichstein, Exper. *10*, 132 (1954); Helv. chim. Acta *37*, 1200 (1954).

[2] Vgl. die methodischen Angaben bei der Tabelle.

[3] S. A. Simpson, J. F. Tait, A. Wettstein, R. Neher, J. v. Euw, O. Schindler und T. Reichstein, Helv. chim. Acta *37*, 1173 (1954).

STUDIORUM PROGRESSUS

Das Strukturbild der Arterienwand unter lebensnahen Präparationsbedingungen und seine physiologische und pathologische Bedeutung

Von S. Hirsch, Bruxelles[1]

Die Histologie der Arterien wird heute häufig als ein abgeschlossenes Forschungsgebiet angesehen, auch in dem Sinne, dass manche der Auffassung sind, dass die Zeit des fixierten histologischen Dauerpräparates hinter uns liegt und dass die Zukunft der Lebendbeobachtung von Feinstrukturen gehört. Indessen zeigt sich gerade bei der Kreislaufforschung, wo die Beobachtung am Lebenden seit 30 Jahren, seit den grundlegenden Experimenten von Krogh und Ricker und ihren Schulen[2] eingeführt ist, dass auch diese Methode ihre grossen Mängel hat[3]. Die Beobachtung am Lebenden, so überraschende Einblicke sie in die Funktion des Kreislaufs ermöglicht, ist unvollkommen, solange es nicht gelingt, ihre Ergebnisse mit einem unter lebensnahen Präparationsbedingungen hergestellten Dauerpräparat zu konfrontieren.

Es ist im allgemeinen sehr schwierig, zu entscheiden, ob das Strukturbild eines Gewebes oder Organs lebensnahen Bedingungen entspricht. Im Falle der Blutgefässe erscheint es *a priori* fast unmöglich, solche Bedingungen zu erlangen. Wir denken hier nicht nur an den Einfluss der Autolyse, der Totenstarre und der durch den Fixierungsakt bedingten Strukturänderungen, der sich bei allen Geweben nach dem Tode geltend macht. Tatsächlich ist die Wand der Blutgefässe mit dem Eintritt des Todes einem dreifachen mechanischen Trauma unterworfen; es wird ausgelöst durch das plötzliche Aufhören der rhythmischen Pulsation, durch die Blutgerinnung und den Leerlauf der Lumina. Indessen ergibt sich gerade aus diesen besonderen Schädigungsfaktoren ein einwandfreier und relativ einfacher *Test* für die Entscheidung, ob das histologische Bild eines Blutgefässes lebensnahen Präparationsbedingungen entspricht. Es gibt beim Lebenden keine blutleeren Arterien; das leere Arterienlumen ist ein Artefakt. Infolgedessen ist man berechtigt, das Vorliegen lebensnaher Bedingungen anzunehmen, wenn auf einem histologischen Schnitt die *Lumina kleiner Blutgefässe mit nichtgeronnenem Blut*, das heisst, mit nicht wesentlich deformierten Blutkörperchen angefüllt sind (Abb. 1 und 2).

Es ist klar, dass ein solcher Befund bei Material, das von in üblicher Weise durchgeführten Autopsien, Operationen und Tierversuchen stammt, nur ausnahmsweise zu erheben ist. Sicherlich sind für die Bedürfnisse der pathologisch-anatomischen Praxis die üblichen Methoden ausreichend; indessen sollte als Grundlage für das Studium von Einzelheiten der Wandstruktur und der Beziehungen zwischen Struktur und Funktion der Arterienwand nur Material dienen, das lebensnahen Präparationsbedingungen entspricht. Nachdem unsere Aufmerksamkeit schon vor längerer Zeit (1941) in einem

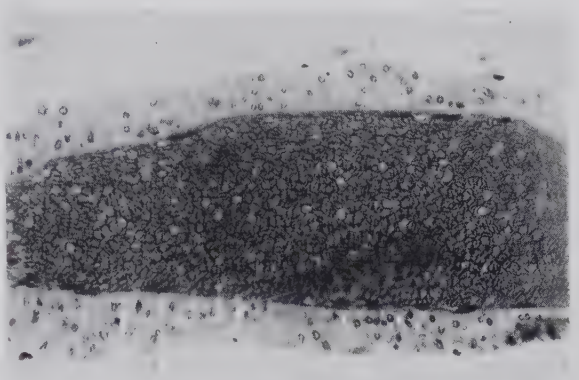


Fig. 1. Kleiner Ast der Koronararterie eines Hundes unter lebensnahen Präparationsbedingungen. Das Lumen der Arterie ist vollkommen mit nichtgeronnenem Blut angefüllt. Die Verhältnisse auf dem fixierten Dauerpräparat entsprechen denjenigen bei der Lebendbeobachtung der Blutströmung in der terminalen Strombahn in transparenten Organen. – Technik: Fixation in Bouin-Hollande, Paraffinschnitt, Hämatoxylin-Eosin-Färbung.

besonderen Falle auf den Test der Blutfüllung gerichtet wurde, haben wir uns seitdem bemüht, durch eine Technik, deren Einzelheiten an anderer Stelle beschrieben

Fig. 2. Kleiner Ast der Koronararterie eines Hundes unter lebensnahen Präparationsbedingungen. Gleiches Präparat wie Figur 1: Darstellung des elastischen Gewebes mittels kombinierter Resorzin-Fuchsin-Hämatoxylin-Färbung. Linienförmige Anordnung der elastischen Elemente.

sind[1], Strukturbilder von menschlichen und tierischen Arterien zu erhalten, die optimalen, das heisst lebensnahen Präparationsbedingungen entsprechen[2] (Abb. 3).

[1] Laboratoire d'Anatomie pathologique de l'Université de Bruxelles.

[2] A. Krogh, J. Physiol. *52*, 409 (1919); *53*, 399 (1920); *The Anatomy and Physiology of Capillaries* (New Haven, 1929). – G. Ricker, *Pathologie als Naturwissenschaft* (Berlin 1924).

[3] Es genügt, an die terminologischen Schwierigkeiten zu erinnern, an die Unbestimmtheit der Begriffe kleinste Arterie, Arteriole, Präkapillare, an die Feststellung kontraktiler Endothelzellen, mit Muskulatur ausgestatteter kleinster Venen und präkapillarer Sphinkter. Vgl.: B. Vimtrup, Z. Anat. Entwicklungsgesch. *65*, 150 (1922). – R. Chambers und B. W. Zweifach, Amer. J. Anat. *75*, 173 (1944). – J. Roskam, *L'hémostase spontanée* (Paris 1951). – L. Illig, Klin. Wschr. *31*, 366 (1953). – J. Hugues, Arch. intern. Physiol. *61*, 565 (1953).

[1] S. Hirsch, Acta biol. belg. *1*, 364 (1941); Cardiologia *6*, 1, 105 (1942); Arch. Biol. *53*, 513 (1942); Schweiz. med. Woch. *75*, 539 (1945); Arch. Malad. Cœur *40*, 433 (1947); Acta Anatomica *8*, 168 (1949); Acta med. Scand. *138*, 449 (1950); I^er^ Congr. mond. Cardiol. Paris, C. r. *1*, 601 (1951).

[2] Es spricht für den Wert des Blutfüllungstests, dass auf Präparaten, die ihm genügen, besonders empfindliche Strukturen, die sich der Darstellung bei der üblichen Technik meist entziehen, wie Lamellenkörperchen und die Wände der kleinen Lymphgefässe, regelmässig nachweisbar sind.

Am Rande der Lumina kleiner Arterien, die mit nichtgeronnenem Blut angefüllt sind, sieht man häufig Vakuolen; sie kündigen den bevorstehenden Eintritt der Koagulation an. Wenn die Fremdkörperwirkung des absterbenden Endothels die Koagulation begünstigt, so

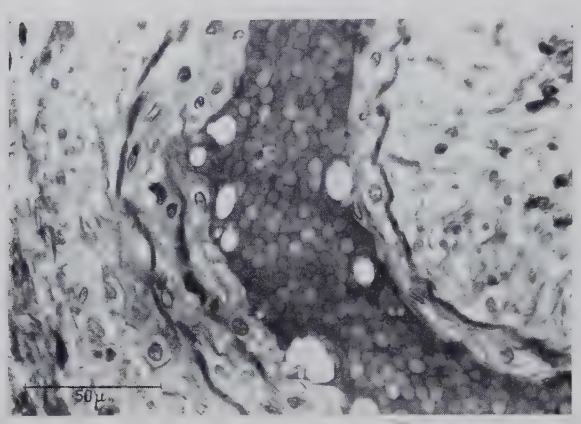


Fig. 3. Kleine Arterie aus der Coccygealgegend des Menschen unter lebensnahen Präparationsbedingungen. Das Lumen der Arterie ist mit nichtdeformierten Blutkörperchen vollkommen angefüllt. Die an den Rändern des Lumens sichtbaren Gasblasen zeigen an, dass die Koagulation unmittelbar bevorsteht. Im linken unteren Sektor der Wand hyaliner Degenerationsherd. Sektionspräparat (62jährige Frau). – Technik: Fixation in Bouin-Hollande, Paraffinschnitt, Massonfärbung.

schädigt andererseits der Koagulationsprozess das Endothel. Abgesehen von ganz seltenen pathologischen Fällen, ist unter optimalen Bedingungen das Endothel stets intakt. Einrisse sind Artefakte, die durch Gerinnung, Leerlauf oder Fixierung bedingt sind. Unter normalen Verhältnissen haben sich bisher Anhaltspunkte für das

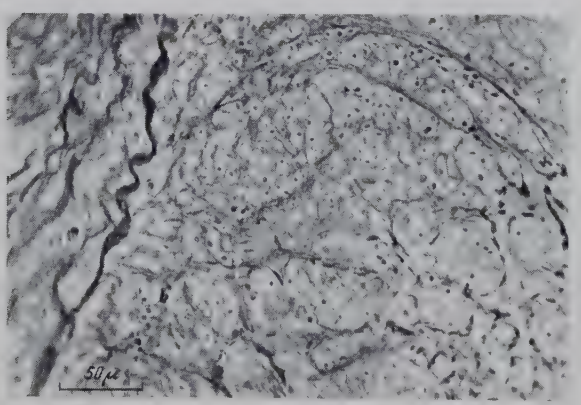


Fig. 4. Wandsektor eines grösseren Astes der Arteria femoralis des Menschen unter lebensnahen Präparationsbedingungen. Man erkennt die Grenzen der einzelnen Muskelfasern, die hell erscheinen und in verschiedener Richtung zur Achse des Gefässlumens orientiert sind. – Technik: Fixation in Bouin-Hollande, Paraffinschnitt, Kombinierte Hämatoxylin-Resorzin-Fuchsin-Färbung.

Bestehen einer besonderen subendothelialen Schicht in der Wand kleiner Arterien nicht ergeben.

Ganz besonderes Interesse beansprucht die Darstellung des *Muskelgewebes* in der Wand der kleinen Arterien. Auf den klassischen Querschnittsbildern der histologischen Lehrbücher sind die Muskelfasern ringförmig angeordnet; die Grenzen der einzelnen Fasern sind nicht sichtbar, und die Orientierung über Lage und Anzahl der Fasern erfolgt an Hand der Zellkerne[1]. Auf dünnen Schnitten eines unter lebensnahen Bedingungen hergestellten Präparates erkennt man die Grenzen der einzelnen Fasern und man sieht relativ häufig neben zirkulär angeordneten, längsgerichtete Fasern, oft als eingesprengte Bündel (Abb. 4). Die längsgerichteten Fasern haben eine mehr oder weniger viereckige, an Epithelzellen erinnernde Form und erscheinen bei allen Färbungen wesentlich heller als die ringförmig angeordneten Fasern. Die hellen Muskelzellen der Arterienwand, die als Quellzellen, «epitheloide Zellen», afibrilläre Muskelzellen, Leiomyoblasten bezeichnet wurden, sind in der

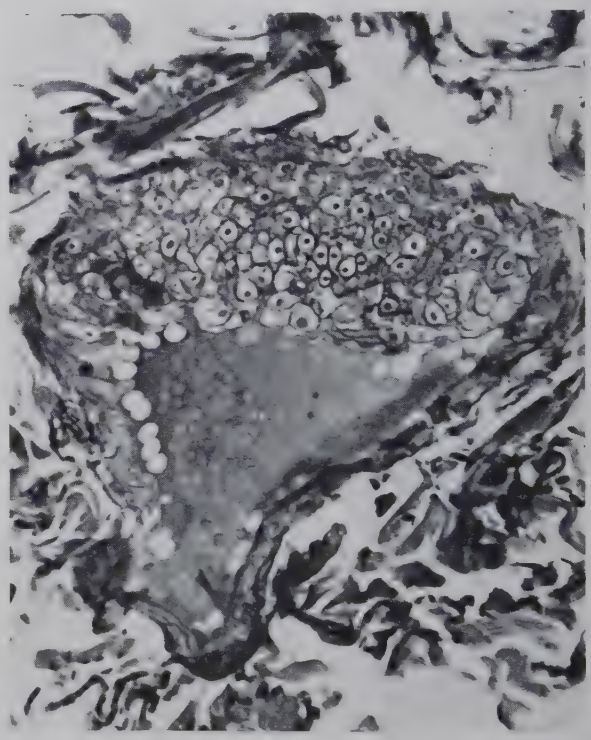


Fig. 5. Kleiner Seitenast im Gebiet der Femoralis des Menschen unter lebensnahen Präparationsbedingungen. Die Beschaffenheit der Blutkörperchen und die zahlreichen Gasblasen am Rande des Lumens zeigen, dass die Koagulation einzusetzen beginnt. Ein Teil der Arterienwand ist aus hellen, epithelartig erscheinenden Muskelzellen zusammengesetzt. – Man kann sich fragen, wie diese sogenannte Polsterbildung nach Abschluss des Gerinnungsprozesses und unter den Folgen der Totenstarre und Autolyse sich darstellen und interpretiert würde. – Technik: Fixierung in Bouin-Hollande, Paraffinschnitt, Hämatoxylin-Eosin-Färbung.

neueren Literatur über die Glomus, die arteriovenösen Anastomosen und die sogenannten Sperrarterien Gegenstand lebhafter Diskussion[2]. Während ältere Autoren sie als pathologische Zellformen betrachteten, ist man

[1] Auf die Unzulänglichkeit und Einseitigkeit mancher Schlussfolgerungen aus dem klassischen Querschnittsbild weist auch der auf diesem Gebiet besonders erfahrene A. Benninghoff hin (Möllendorfs Handb. mikroskop. Anat., VI, 1, Berlin 1930).

[2] Hinsichtlich der Literatur wird auf die neuesten Veröffentlichungen von J. Staubesand, Acta Anatomica *19*, 105, 209, 309 (1953); G. Conti, Acta Anatom. *18*, 234 (1953); A. Bohle, M. Kohler und U. Tomsche, Btr. pathol. Anat. *113*, 414 (1953); C. A. Wagenvoort, Diss. Utrecht (1952), verwiesen.

neuerdings geneigt, ihnen eine endokrine Funktion zuzuschreiben[1] (Abb. 5).

Das *elastische Gewebe* kleiner und kleinster Arterien zeigt sich unter den üblichen Präparationsbedingungen als ein zickzackförmig um das Lumen angeordnetes Band. Unter lebensnahen Bedingungen bilden die elastischen Elemente eine Linie; auf dem Querschnitt eine Kreislinie, auf dem Längsschnitt eine Gerade. Jede Abweichung von der Linienform – von leichten Wellungen, bis zur Zickzack- und Girlandenform, von tiefen Einschnürungen bis zur Aufsplitterung in kleine Striche und Punkte – entspricht einer Einschränkung oder Aufhebung der elastischen Funktion, die verschiedene Ursachen haben kann. Sie kann durch den Stillstand der Blutströmung im Augenblick des Todes hervorgerufen sein; dann handelt es sich um einen Artefakt. Das gilt für die meisten Zickzackfiguren um blutleere Arterienlumina. In anderen Fällen weist die Abweichung von der Linienform auf den im Augenblick der Fixierung bestehenden Funktionszustand der lebenden Arterienwand hin; sie kann mit der passiven pulsatorischen Dehnung oder bei den kleinen Arterien mit der aktiven Leistung der Arterienwand zusammenhängen[2]. Diese Schlussfolgerungen ergeben sich aus dem systematischen Studium der eigenartigen Beziehungen zwischen muskulären und elastischen Elementen in der Wand der sogenannten Polster- oder Drosselarterien[3]. Es zeigt sich, dass wenn in einem Wandsektor klare sogenannte «epitheloide» Muskelzellen nachweisbar sind, die elastischen Elemente aufgesplittert als Striche und Punkte zwischen den Muskelzellen sichtbar sind; auf der anderen Seite sind beim Vorhandensein eines zusammenhängenden elastischen Bandes helle Muskelzellen nicht nachweisbar. Indessen ist diese eigenartige Beziehung zwischen elastischen und muskulären Elementen nicht nur in den kleinen Drosselarterien, sondern unter optimalen Präparationsbedingungen vor allem auf Längs- und Tangentialschnitten auch in der Wand mittlerer Arterien gelegentlich nachweisbar. Der Schlüssel zur Deutung dieser Erscheinung ergab sich durch Experimente an Hunden, deren Herzen während der Einwirkung von Adrenalin schlagend in die Fixierungsflüssigkeit eingelegt waren. In der Wand zahlreicher kleiner Arterien mit verengtem oder verschlossenem Lumen bestand die gleiche eigenartige Beziehung zwischen muskulären und elastischen Elementen wie in den sogenannten Sperrarterien des Menschen[4]. Wenn man in Betracht zieht, dass sich im Vorgang der Vasokonstriktion ein Antagonismus zwischen Kontraktilität und Elastizität der Arterienwand geltend macht, so liegt der Gedanke nahe, dass das geschilderte Phänomen das *morphologische Äquivalent einer vasokonstriktorischen Aktion* ist; die Gegenwart heller Muskelzellen bei Aufsplitterung der elastischen Elemente entspricht dem sich schliessenden, das Verschwinden der Muskelzellen und die Annäherung der elsatischen Elemente an die Linienform dem sich öffnenden Arterienlumen (Abb. 6).

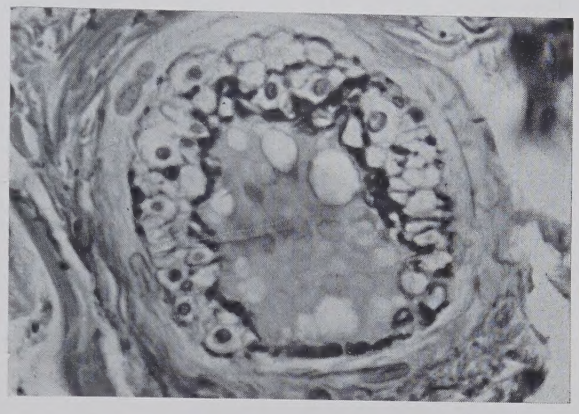


Fig. 6. Kleine Arterie mit sogenannter Sperrvorrichtung aus dem subepikardialen Fettgewebe eines 54jährigen Mannes. Lebensnahe Präparationsbedingungen. Das Gefässlumen ist im oberen Teil des Bildes durch helle «epitheloide» Muskelzellen (Quellzellen) verschlossen. Demonstration der eigenartigen Beziehung zwischen muskulären und elastischen Elementen der Arterienwand: Dort, wo die Muskelzellen sichtbar sind, erscheinen die elastischen Elemente zwischen den Zellen als einzelne Striche und Punkte; im unteren Sektor, wo die Elastika eine zusammenhängende Linie bildet, sind keine Muskelzellen sichtbar. (Vgl. S. Hirsch, *The Structural Mechanism of Arterial Contraction*, Cardiologia *21*, 65 [1952]). – Technik: Fixierung in Bouin-Hollande, Paraffinschnitt, Hämatoxylin-Pikrofuchsin-Orcein-Färbung.)

Aus diesen und ähnlichen Feststellungen ergibt sich die Bedeutung von histologischen Untersuchungen der Arterienwand unter lebensnahen Bedingungen. In der Tat erscheinen sie geeignet, eine Anzahl zur Zeit bestehender Differenzen zwischen anatomisch-mikroskopischen und auf Lebendbeobachtung beruhenden hämodynamischen Kriterien zu beseitigen und eine bessere Übereinstimmung der Resultate morphologischer, physiologischer und physiopathologischer Kreislaufforschung herbeizuführen. Als Beispiel erwähnen wir die Frage des Bestehens von Ring- und Längsmuskelfasern in der Arterienwand. Die kinematographische Beobachtung kleiner und kleinster Arterien beim Lebenden in der terminalen Strombahn zeigt, dass die Strukturelemente der Wand fast in keinem Augenblick in der gleichen Ebene fixiert sind. Sie sind durch von innen und von aussen auf die Wand einwirkende mechanische Einflüsse bald zirkulär, bald längs, bald schräg zur Achse des Lumens gerichtet. Es gibt somit beim Lebenden keine besonderen Ring- und Längsmuskelfasern, sondern die Wandmuskulatur ist eine Einheit. Es ist die *Plastizität* und *Aktivität* der einheitlichen Muskulatur, die es bedingt, dass auf dem unter lebensnahen Präparationsbedingungen hergestellten histologischen Schnitt die Muskelfasern oft in verschiedener Richtung getroffen sind. Im Zusammenhang mit dieser Feststellung erscheint eine Revision in der Frage der sogenannten arteriellen Sondervorrichtungen notwendig. Es ist anzunehmen, dass es sich bei den als Polster, Sphinkter und Ventile beschriebenen Bildungen nicht um permanente Strukturen, sondern um *fixierte Momentbilder*

[1] J. Mollard und C. Regaud, Congr. méd. Lille (1899), und M. Rabe, Presse méd. *10*, 927 (1902), berichten über Arterien mit «Epithelisierung» und «Vacuolisierung» der Media. Die endokrine Theorie wird vor allem von S. v. Schumacher, Z. mikroskop. Anat. *43*, 107 (1938); N. Goormaghtigh, *La fonction endocrine des artérioles rénales*, Louvain (1944); und F. W. Dunihue, Arch. Pathol. *32*, 211 (1941) vertreten.

[2] A. Benninghoff, Z. Zellforsch. *6*, 348 (1928), stellte durch das Spaltverfahren fest, dass in der Wand der grossen Arterien die Muskelfasern als eine Art Spannmuskeln schräg an den elastischen Elementen ansetzen. Es ist bemerkenswert, dass es ihm nicht gelang, die gleiche Beziehung bei den kleinsten Arterienästen nachzuweisen, und führte das auf den Fortfall der pulsatorischen Dehnung zurück.

[3] S. Hirsch, Acta biol. belg. *1*, 364 (1941); Cardiologia *6*, 1, 105 (1942); Arch. Biol. *53*, 513 (1942); Schweiz. med. Woch. *75*, 539 (1945); Arch. Malad. Cœur *40*, 433 (1947); Acta Anatomica *8*, 168 (1949); Acta med. Scand. *138*, 449 (1950); I^er Congr. mond. Cardiol. Paris, C. r. *1*, 601 (1951).

[4] Vgl. S. Hirsch, C. r. Soc. biol. *146*, 618 (1952); Presse méd. *60*, 417 (1952); Cardiologia *21*, 65 (1952).

eines in Bewegung befindlichen Teils der Wandmuskulatur handelt.

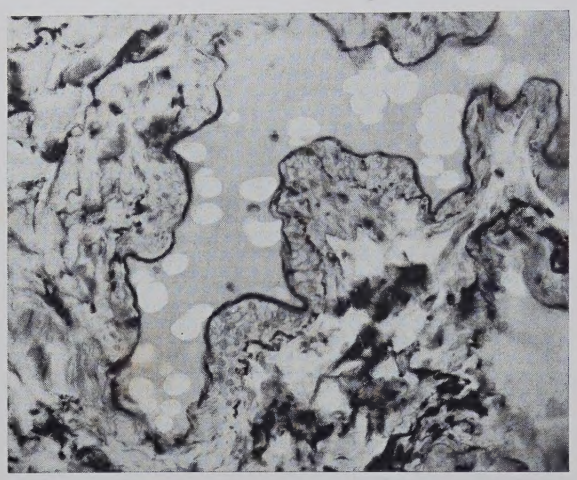


Fig. 7. Längsschnitt durch eine kleine Arterie der Femoralgegend des Menschen. Im Lumen sind ausser Erythrocyten und den Beginn der Koagulation anzeigenden Gasblasen einige Leukocyten erkennbar. Das Bild zeigt, dass unter Verhältnissen, die der Lebendbeobachtung angenähert sind, die kleinen Arterien keine starren Röhren sind, sondern dass sich in der Profilierung der Wand die Plastizität und Aktivität der Wandmuskulatur ausdrückt. Man beachte die in verschiedenen Sektoren deutliche Klarheit des Sarkoplasmas und die charakteristische Anordnung der elastischen Elemente. Technik: Fixierung in Bouin-Hollande, Paraffinschnitt, Kombinierte Färbung Hämatoxylin-Resorzin-Fuchsin.

Was den Unterschied zwischen hellen und dunklen Muskelfasern (Abb. 7) betrifft, so legen unsere Beobachtungen den Gedanken nahe, ihn mit dem Funktionszustand bzw. dem Tonus der Faser in Beziehung zu bringen. Bei den hellen Fasern dürfte es sich um solche Elemente handeln, die von der Fixation im Zustand der Aktivität gewissermassen überrascht wurden. Man findet helle Muskelzellen häufiger auf Längsschnitten als auf Querschnitten, weil sich auf ersteren die bei der Durchschneidung des zylindrischen Arterienrohrs entstehenden Spannungsänderungen stärker bemerkbar machen als beim Querschnitt. Indem wir die hellen Fasern als «*aktive*» und nicht etwa als kontrahierte Elemente bezeichnen, tragen wir der Unsicherheit der Kriterien zwischen Kontraktion und Tonus der Muskelfaser Rechnung[1]. Hier berühren unsere Feststellungen das immer noch sehr umstrittene Problem der Funktion der glatten Muskelfaser. Alle auf diesem Gebiet erfahrenen Forscher betonen die Verwirrung, die dadurch hervorgerufen wird, dass sowohl Physiologen wie Morphologen oft die glatte Muskelfaser als eine Art vereinfachter quergestreifter Faser auffassen[1]. Als Beispiel verweisen wir auf die Tatsache, dass in der pathologisch-anatomischen Literatur nicht selten von einer Hypertrophie der Arterienmuskulatur die Rede ist; man ist sich nicht klar darüber, dass der Begriff der *Arbeitshypertrophie* nur auf quergestreifte Muskulatur anwendbar ist. Wenn bei der Aktion der quergestreiften Muskelfaser die *Verkürzung* im Vordergrund steht, so macht sich bei der Aktion der glatten Faser vor allem ein *Quellungsvorgang* geltend. Das Studium des Strukturbildes der Arterienwand unter lebensnahen Präparationsbedingungen kann eine morphologische Stütze bieten für die sogenannte Quellungstheorie der glatten Muskulatur.

[1] Siehe hierzu die Diskussion über «aktive» Muskelfasern in der älteren anatomischen Literatur: B. Henneberg, Anat. Hefte *1*, 54, 427 (1901). – G. Roskin, Arch. Zellforsch. *17*, 368 (1923).

Summary

At first sight it seems impossible to obtain a picture of arterial structure fixed in the true conditions of life. The arterial wall particularly is exposed to severe forces of alteration at the moment of death. Even before autolysis and rigor mortis, it is submitted to a triple trauma: the sudden cessation of rhythmical pulsation, the emptying of the lumina and the blood coagulation. Nevertheless precisely, these factors give us a simple and relatively safe *test* for deciding whether a histological preparation corresponds to the true life conditions; we may assume this to be so when the lumen of small blood vessels is full of *uncoagulated* blood. Studies on such preparations show that during life there are no particular annular and longitudinal muscle fibres in the arterial wall, it is rather the *plasticity and activity of a unitary musculature* which determines the appearance of variously oriented fibers in histological preparations. As for the elastic tissue, it appears as a continuous line, when in activity; curvatures or ruptures mean a limitation or suppression of the elastic function. Observations on the so-called regulating apparatus in arteries of man, completed by others on dogs under influence of adrenalin, lead one to consider the particular relationship of clear muscle cells and elastic elements as the *morphological equivalent of vasoconstrictive action*. These results call for a revision of some histological, histopathological and even physiological concepts, such as the accepted histological views on the existence of arterial closure apparatus. The aspects described as sphincters, "Polster", "bourrelets", valves are not permanent structures but *snapshots of moving parts of the arterial wall*. The clear muscular cells named "epitheloid cells", "Quellzellen", Leiomyoblasts are muscle fibers quasi surprised by the fixation in the *active state*.

[1] Vgl. O. v. Fürth, Ergebn. Physiol. *17*, 363 (1919). – L. Lapicque, Traité Physiol. *8*, 1 (Paris 1929). – A. Szent-György, *Chemistry of muscular contraction* (New York 1951).

Die Forschungslaboratorien der Sandoz A.G. haben einen entscheidenden Anteil an der Reindarstellung und Konstitutionsaufklärung der herzwirksamen Glykoside und leisteten wesentliche Beiträge zu deren pharmakologischen Erforschung.

Aus diesen grundlegenden Arbeiten resultierten für die Therapie der Herzkrankheiten folgende Spezialpräparate:

Acylanid

Cedilanid

Digilanid

Scillaren

Strophosid

SANDOZ

SANDOZ A.G.

Elemente der Mathematik vom höheren Standpunkt aus

Diese Bandreihe bringt die Grundlagen ausgewählter Kapitel der mathematischen Wissenschaften in einer Form, die nur wenige Vorkenntnisse erfordert und die wesentlichen Ideen hervortreten lässt. Es werden in freier Wahl einige der schönsten Gebiete der Mathematik ihren Grundgedanken nach in sorgfältig bearbeiteten, leicht zugänglichen und im Umfang mässigen Überblicken dargeboten.

In Kürze erscheint Band IV:

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Es werden Eigenschaften geometrischer Figuren, nämlich «Waben» im Kleinen untersucht, die bei beliebigen Punktabbildungen erhalten bleiben. Dabei ergeben sich Beziehungen insbesondere zur Normographie der Techniker und zur Algebraischen Geometrie. Auf Anschaulichkeit, Vielseitigkeit und Verständlichkeit wird Wert gelegt sowie auf manche offene Frage hingewiesen. Eine Anzahl von Figuren belebt den Text.

Aus dem Vorwort:

«Im Laufe meines Lebens, habe ich im Zusammenwirken mit Schülern und Mitarbeitern verschiedene Gärtchen gehegt, und eines davon trägt die Aufschrift «Geometrie der Gewebe». Es hat mir seltsame Früchte getragen, wie etwa eine Einladung zu einer Tagung von Textilfachleuten. Deshalb spreche ich statt von «Geweben» jetzt lieber von «Waben», da mir Beziehungen zu Bienen und Imkern anziehender zu sein scheinen als zu Webern. – Wenn Francesco Severi kürzlich schrieb: «Lamatematica moderna è ammalata di astrattismo», so darf ich für unseren Zweig der Geometrie ein grosses Mass von Gesundheit (oder Mangel an Moderne?) in Anspruch nehmen, da es sich hier meist um sehr ‚anschauliche' Fragen handelt.»

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